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HANDBOOK of RECEPTORS and CHANNELS

LIGAND- and VOLTAGE-GATED ION CHANNELS

Edited by

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Voltage-Gated Potassium Channel Genes

K. George Chandy and George A. Gutman

2.1.0 Introduction

2.1.0.1 Potassium Channels

Potassium channels play a vital role in the functioning of diverse cell types (Rudy, 1988; Hille, 1993). They regulate membrane potential in electrically excitable cells such as nerves and muscle, as well as in nonexcitable cells such as lymphocytes (Hille, 1993). Although many vertebrate K^+ channel genes have been isolated by molecular cloning (see Gutman and Chandy, 1993), and a variety of experimental strategies have defined functional domains within the channel proteins (see Miller, 1990, 1992; Jan and Jan, 1992), little progress has been made in the biochemical characterization of these proteins. In addition, little effort has been made to define the mechanisms that control transcription and functional expression of these important molecules. Several excellent reviews have dealt with the structural features that underlie particular biophysical and pharmacological properties of K^+ channels (Miller, 1990; 1992; MacKinnon, 1991a; Jan and Jan, 1992; Pongs, 1992, 1993), and others discuss the *Shaker* and related K^+ channel genes in flies (Salkoff et al., 1992; Pongs, 1993), the mammalian Isk K^+ channel (Swanson et al., 1993), and the calcium-activated K^+ channel (Garcia et al., 1993). This chapter will focus on the mammalian voltage-gated K^+ (Kv) channel gene family. We discuss their amino acid sequence alignments and evolutionary relationships, compare their electrophysiological and pharmacological properties, and discuss possible mechanisms that may underlie their tissue-specific expression.

2.1.0.2 The Extended Kv Channel Gene Family

Early in 1987, three groups, using genetic techniques in combination with molecular strategies, isolated the gene encoded by the *Shaker* (Sh) locus in

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Drosophila melanogaster (Kamb et al., 1987; Papazian et al., 1987, Schwarz et al., 1988; Pongs et al., 1988). Three related fly genes, *Shab, Shaw,* and *Shal,* were soon isolated, and each of these produced functionally distinct channels (Butler et al., 1989, 1990; Wei et al., 1990; Covarrubias et al., 1991). A topology has been proposed for this family of channels (shown in Figure 1) on the basis of hydropathy plots and structure–function analyses (Miller, 1990, 1992; Jan and Jan, 1992; Pongs, 1992; Durell and Guy, 1992). The channel is thought to have six membrane-spanning segments, termed S1 through S6, with both the amino- and carboxy-termini located intracellularly. The region between the S5 and S6 segments (the "P-region," or "pore") is thought to participate in forming the ion conduction pathway, while the S4 segment forms a major part of the voltage sensor (Miller, 1990; Papazian et al., 1991; Jan and Jan, 1992; Pongs, 1992; Durell and Guy, 1992). The loop linking S1 and S2 has recently been experimentally confirmed to be located extracellularly (Chua et al., 1992; Shen et al., 1993). A more detailed description is provided below.

Using *Shaker* probes, Tempel et al. (1988) and Baumann and colleagues (1988) isolated the first mammalian homologues of the *Shaker* gene. In the next four years a total of 17 vertebrate genes encoding voltage-gated K^+ channels were isolated from mammals and frogs. The current status of this

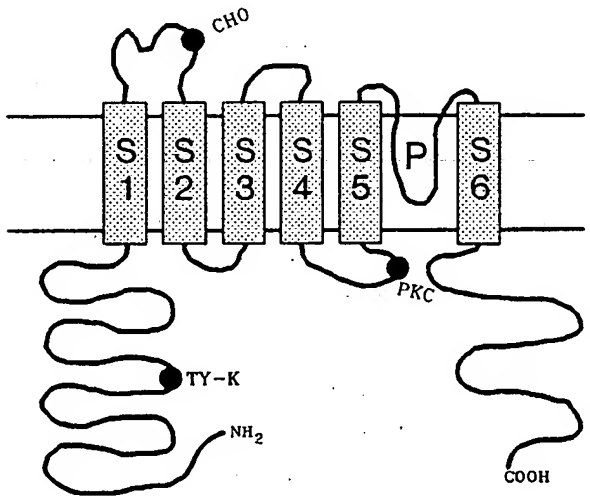

FIGURE 1. Schematic diagram of the presumed structure of voltage-gated K^+ channels. The six putative membrane-spanning domains are labeled S1 through S6, and "P" indicates the P-region, which is thought to form part of the ion conducting pore. Three sites of possible posttranslational modification are indicated, an N-glycosylation ("CHO") and a tyrosine kinase ("TY-K") site both present in mammalian *Shaker*-subfamily channels, and a protein kinase C ("PKC") site present in all known voltage-gated K^+ channels (see text).

continuously growing list is shown in Table 1, which includes the assigned gene names conforming with the recently adopted standardized nomenclature (Chandy et al., 1991). Most of these genes segregate into four clearly defined gene subfamilies (Chandy et al., 1991; Gutman and Chandy, 1993; see references in Table 1), each of which (Kv1.1-1.8, Kv2.1-2.2, Kv3.1-3.4, Kv4.1-4.3) is structurally and evolutionarily related to one of the four fly genes *(Shaker, Shab, Shaw,* and *Shal).* Two additional mammalian genes have been isolated (IK8/Kv5.1 and K13/Kv6.1) for which fly homologues have not yet been identified (Drewe et al., 1992); their inclusion in this scheme of nomenclature should be treated with caution, as their functionality and voltage dependence have yet to be confirmed by expression of the cloned sequences. In addition, two *Shaker* homologues from *Xenopus* (XSha1, XSha2) have been described and have been incorporated into this standardized nomenclature. *Shaker* homologues have also been described in *Aplysia* (Pfaffinger et al., 1991), in *C. elegans* (Wei et al., 1991), in leeches (Johansen, 1990), and in schistosomes (E. Kim, personal communication). The incorporation of the *Aplysia* channel (Shen et al., 1993) into the standardized nomenclature is currently questionable, as their divergence appears to predate the gene duplications that gave rise to the multimembered Kv gene subfamilies in mammals and frogs (discussed below).

2.1.1 *Shaker*-Related Gene Subfamily

2.1.1.1 Protein Sequence Comparisons

Figure 2 shows the alignment of the amino acid sequences of all known *Shaker* homologues, including those of humans, rats, mice, dogs, cattle, frogs, and the mollusc *Aplysia,* together with the *Drosophila Shaker* sequence.* An obvious feature of this alignment is the striking sequence conservation within certain regions, including most of the hydrophobic core

* In discussing this and other sequence alignments, we would emphasize several cautionary notes:

1. Sequences from less than full-length mRNA can obscure authentic start sites (e.g., DRK1), or suggest spurious polyadenylation sites (e.g., MBK1).
2. In the case of protein-coding sequences determined from genomic DNA alone, lacking mRNA sequences with which to compare them, the possible presence of unidentified introns may obscure the sites of translation initiation or termination.
3. The presence of unspliced or only partially spliced mRNAs, which appears to be not uncommon among K+ channels, may mask the presence of introns (e.g., MBK1) and also obscure translational start and stop sites (e.g., *Shaker).*
4. Not surprisingly, a variety of unresolved sequencing errors exists in published K+ channel sequences. While many may be of no major consequence, several cause local shifts in the reading frame (e.g., mKv3.3 vs rKShIIID, and RCK4 vs RHK1), or alter either translational start sites (mKv3.3 vs rKShIIID) or termination sites (RK5 vs Shal1).

In our discussion, we have attempted to avoid drawing conclusions from sequence differences that are likely to be the result of technical errors, and to rely largely on features that can be confirmed by comparison between independently determined sequences of the same gene, or of homologous genes between different species.

Table 1a. Nomenclature of Mammalian Voltage-Dependent Potassium Channel Genes[a]

Standard names[b]	Names in use[c]			
	Mouse	Rat	Human	(Chrom.)
Shaker-related subfamily 1				
Kv1.1 (KCNA1)	MBK1[1] MK1[5,17]	RCK1[2] RBK1[7] RK1[17,27]	HuK(I)[3,4,41] HKC-1[71]	(12p13[35,45,62,80])
Kv1.2 (KCNA2)	MK2[5] MK5[17]	BK2[6] RCK5[9] NGK1[10] RK2[27] RAK[40] RH1[72]	HuK(IV)[3,4,41]	
Kv1.3 (KCNA3)	MK3[5,11,17]	RCK3[9] RGK5[13] KV3[14]	HuK(III)[3,4] hPCN3[12] HLK3[31] HGK5[32]	(1p13.3[31]) (1p21[59])
Kv1.4 (KCNA4)	MK4[17] mKv1.4[80]	RCK4[9] RHK1[15] RK4[17,27] RK8[17]	HuK(II)[3,4,41] hPCN2[12a] HK1[30]	(11q13-14[57]) (11p14.1[58,80])
Kv1.5 (KCNA5)[d]	—	KV1[14] RK3[27] RMK2[36]	HuK(VI)[3,4] hPCN1[12] HK2[30] HCK1[33] fHK[73]	(12p[33,45])
Kv1.6 (KCNA6)	MK2[17] MK6[56]	RCK2[16] KV2[14]	HBK2[16] HuK(V)[3,4]	
Kv1.7 (KCNA7)	MK6[17] MK4[18]	RK6[17]	—	(19q13.3[39,45])
Shab-related subfamily 2				
Kv2.1 (KCNB1)	mShab[19]	DRK1[20]	hDRK1[67,68]	
Kv2.2 (KCNB2)	—	CDRK[34]	—	
Shaw-related subfamily 3				
Kv3.1 (KCNC1)[e]	NGK2[10] mShaw22[22]	Kv4[21] Raw2[29]	hKv3.1[35] NGK2-KV4[37,38]	(11p15[35,38]) (11p14.3-15.2[80])
Kv3.2 (KCNC2)[e]	mShaw12[22]	Rshaw12[22] RKShIIIA[23] Raw1[29] rKv3.2b,c[60]	HKShIIIA[37]	(12[35,45]) (19q13.3-13.4[84])
Kv3.3 (KCNC3)[e]	mKv3.3[24] mShaw19[22]	RKShIIID[37]	HKShIIID[37] hKv3.3[44]	(19q13.3-4[24,39,44,45])
Kv3.4 (KCNC4)	mKv3.4[24]	Raw3[25]	HKShIIIC[28]	(1p21[24,28,45])
Shal-related subfamily 4				
Kv4.1 (KCND1)	mShal[26]	—	—	
Kv4.2 (KCND2)	—	RK5[27] Shal1[46]	—	
Kv4.3 (KCND3)	—	RKShIVB[54,55]	—	
Subfamily 5				
Kv5.1 (KCNF1)	—	IK8[43]	—	
Subfamily 6				
Kv6.1 (KCNG1)	—	K13[43]	—	

Table

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3,80)

19,45)

38)
3-15.2[80])

3-13.4[84])

3-4[24,39,44,45])

28,45)

Table 1a. (Continued)

[a] See Chandy et al., *Nature* 352, 26, 1991; Gutman and Chandy, *Sem. Neurosci.* 5, 101–106, 1993.

[b] Human locus names assigned by the Human Gene Mapping Workshop (HGMW) are given in parentheses.

[c] Homologues of Kv1.1, Kv1.5, Kv1.6 and Kv1.7 have been isolated from hamster,[17] two *Shaker*-related genes (*Xsha1* and *Xsha2*) from *Xenopus*,[8,61] homologues of Kv1.2 (BGK5)[69] and Kv1.4 (BAK4)[63] from cattle, Kv1.2 (dKv1.2)[70] from dog, and several clones showing similarity with Kv1.1–1.4 from rabbit.[42] Homologues of the *Drosophila eag* gene have been isolated from mouse (meag), rat (reag), and human (heag), representing at least two distinct members of a cyclic nucleotide-binding K+ channel family.[65,66] Genomic and cDNA sequences encoding the IsK/minK channel, a putative single-membrane-spanning domain channel, have been isolated from rats, mice and humans;[47–49] the human homologue has been assigned the HGMW name KCNE1, and the gene has been localized to human chromosome 21q22.[35,45,64]

[d] Termed "KCNA1" in Reference 33.

[e] Alternately spliced forms are known.

Table 1b. K+ Channel Sequences in GenBank/EMBL Databases[a]

Name[b]	Comments	Access. no.	Ref.
mKv1.1	MBK1	Y00305	1
	MK1	M30439[c]	5
rKv1.1	RCK1	X12589	2
	RBK1	M26161	6,7
hKv1.1	HuK(I)	L02750	3,4,41
xKv1.1	XSha1	M94258[c]	61
mKv1.2	MK2	M30440[c]	5
rKv1.2	BK2	J04731	6
	RCK5	X16003	9
	RAK	M74449	40
hKv1.2	HuK(IV)	L02752	3,4,41
bKv1.2	BGK5[e]	L23170	69
xKv1.2	XSha2	M35664[c]	8
mKv1.3	MK3	M30441[c]	5,11
dKv1.2	dKv1.2	L19740	70
rKv1.3	RCK3	X16001	9
	RGK5	M30312[c]	13
	KV3	M31744[c]	14
hKv1.3	hPCN3	M55515[c]	12
	HLK3	M85217	31
	HGK5	M38217[c]	32
rKv1.4	RCK4	X16002	9
	RHK1	M32867	15
hKv1.4	HuK(II)	L02751	3,4,41
	hPCN2	M55514	12
	HK1	M60450	30
hKv1.4		U037322-3[c]	80
bKv1.4	BAK4	X57033	63
rKv1.5	KV1	M27158[c]	14
hKv1.5	hPCN1	M55513	12
	HK2	M60451	30
	HCK1	M83254[c,d]	33
mKv1.6	MK1.6	M96688	56
rKv1.6	RCK2	X17621	16
	KV2	M27159[c,e,f]	14
hKv1.6	HBK2	X17622	16

Table 1b. (Continued)

Name[b]	Comments	Access. no.	Ref.
mKv2.1	mShab	M64228	19
rKv2.1	DRK1	X16476[g]	20
hKv2.1	hDRK1	L02840[g]	67
	hDRK1	X68302	68
rKv2.2	CDRK	M77482	34
mKv3.1	NGK2	Y07521	10
rKv3.1	KV4	M68880	21
	Raw2	X62840	29
hKv3.1	hKv3.1	M96749[e]	35
rKv3.2[h]	RKShIIIA	M34052	23
	Raw1	X62839	29
	rKv3.2b	M59211	60
	rKv3.2c	M59313	60
mKv3.3	mKv3.3	X60796-7[e]	18,24
rKv3.3[h]	RKShIIID	M84210-1	37
hKv3.3	hKv3.3	Z11585[e,e]	44
mKv3.4	mKv3.4	M81253[e,e]	18,24
rKv3.4	Raw3	X62841	25
hKv3.4	HKShIIIC	M64676	28
mKv4.1	mShal	M64226	26
rKv4.2	RK5	M59980[i]	27
	Shal1	S64320	46
rKv5.1	IK8	M81783	43
rKv6.1	K13	M81784	43
Other Kv channels			
Shaker	*Drosophila*	M17211	50
Shab	*Drosophila*	M32659	51
Shal	*Drosophila*	M32660	51
Shaw	*Drosophila*	M32661	51
KC2...	Rabbit	M81350-4[e,e]	42
APLK	*Aplysia*	M95914	83
IsK (minK) channels			
mIsK	Mouse heart	S57779	49
rIsK	Rat kidney	M22412	47
hIsK	Human	M26685[e]	48
Other K^+ channels			
Eag	*Drosophila*	M61157	52
Slo[h]	*Drosophila*	M69053[e]	53
	Drosophila	M96840	82
mSlo	Mouse	L16912	74
KAT1	*A. thaliana*	M86990	75
AKT1	*A. thaliana*	X62907	76
ROMK1	Rat	X72341	77
IRK1	Rat	X73052	78
GIRK1	Rat	L25264, U09243	85,86
ECOKCH	*E. coli*	L12044	79

[a] cDNA sequences, unless otherwise indicated.
[b] Prefixes to standard names: m, mouse; r, rat; h, human; b, bovine; x, *Xenopus*.
[c] Genomic sequence.
[d] 1 bp insertion causes early termination (compare with HK2, HPCN1).
[e] Partial coding sequence only.

Table 1b.

[f] Full publ
[g] Publishe
[h] Alternate
[i] 2 bp dele

Table 1 ref

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15. Tse
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Fol
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25. Sch
FE
26. Pak
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27. Rol

Table 1b. (Continued)

[f] Full published sequence not incorporated.
[g] Published correction in start site (see Reference 43) not incorporated.
[h] Alternately spliced cDNAs.
[i] 2 bp deletion results in early termination (compare with Shal1).

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Table 1b. (Continued)

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Table 1b. (Continued)

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of the protein (the region that includes the six membrane-spanning domains S1 through S6, and the P or pore-forming region), as well as a substantial portion of sequence on the N-terminal side of S1. Equally striking is the substantial *divergence* of sequences at both ends of the protein, as well as within two regions that are thought to form extracellular loops (between S1/S2 and S3/S4).

2.1.1.1.1 Divergence of N- and C-Terminal Regions; Repetitive Sequences

The extensive sequence divergence at both ends of these proteins suggests either that these regions have very different functions in the different channel isoforms, or that whatever functions they do have depend relatively little on

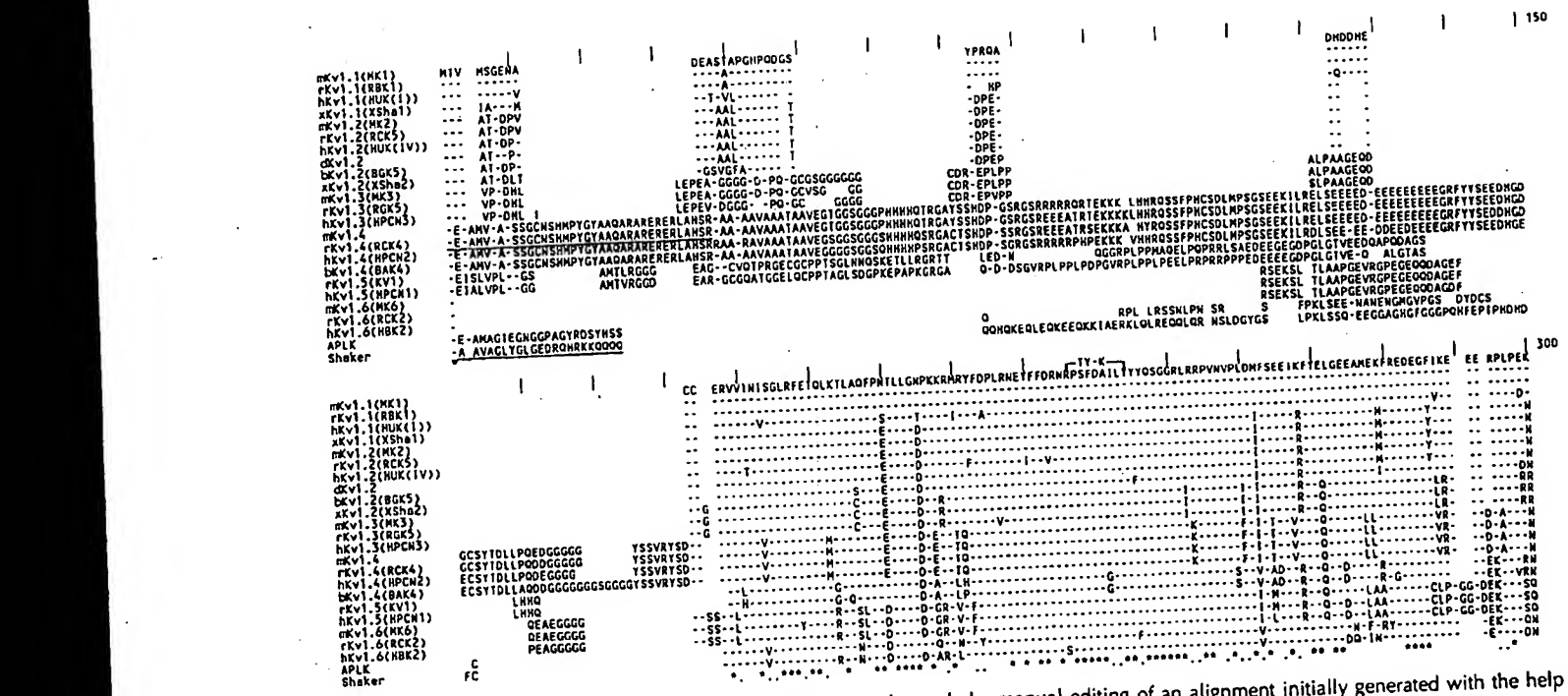

FIGURE 2. Amino acid sequence alignment of *Shaker*-related K+ channels, made by manual editing of an alignment initially generated with the help of the program CLUSTALV (Higgins et al., 1992). Dashes indicate identity with the sequence at the top of the alignment (mKv1.1), and hash marks above the top sequence mark every tenth residue. Below the alignment, an asterisk indicates positions at which all sequences are identical, and a period indicates positions that show only conservative substitutions. Shown by brackets above the alignment are the six putative membrane-spanning regions (S1–S6), the "pore" motif (P), a potential tyrosine kinase recognition sequence (TY-K), which is present in all *Shaker*-related sequences, and two potential protein kinase C sites (PKC), at least one of which is present in *all* voltage-gated K+ channels. Asterisks above the alignment in the region between S1 and S2 mark the positions of potential N-glycosylation sites in one or more of the sequences below, each of which is underlined in the sequence itself. The underlined sequences at the N-terminus of rKv1.4 and *Shaker* represent peptides that have been shown to mediate fast inactivation in these channels; the arrow marks the cysteine residue within this peptide in rKv1.4 whose oxidation removes fast inactivation (see text).

tyrosine kinase recognition sequence (TY-K), which is present in all *Shaker*-related sequences, and two potential protein kinase C sites (PKC), at least one of which is present in *all* voltage-gated K⁺ channels. Asterisks above the alignment in the region between S1 and S2 mark the positions of potential N-glycosylation sites in one or more of the sequences below, each of which is underlined in the sequence itself. The underlined sequences at the N-terminus of rKv1.4 and *Shaker* represent peptides that have been shown to mediate fast inactivation in these channels; the arrow marks the cysteine residue within this peptide in rKv1.4 whose oxidation removes fast inactivation (see text).

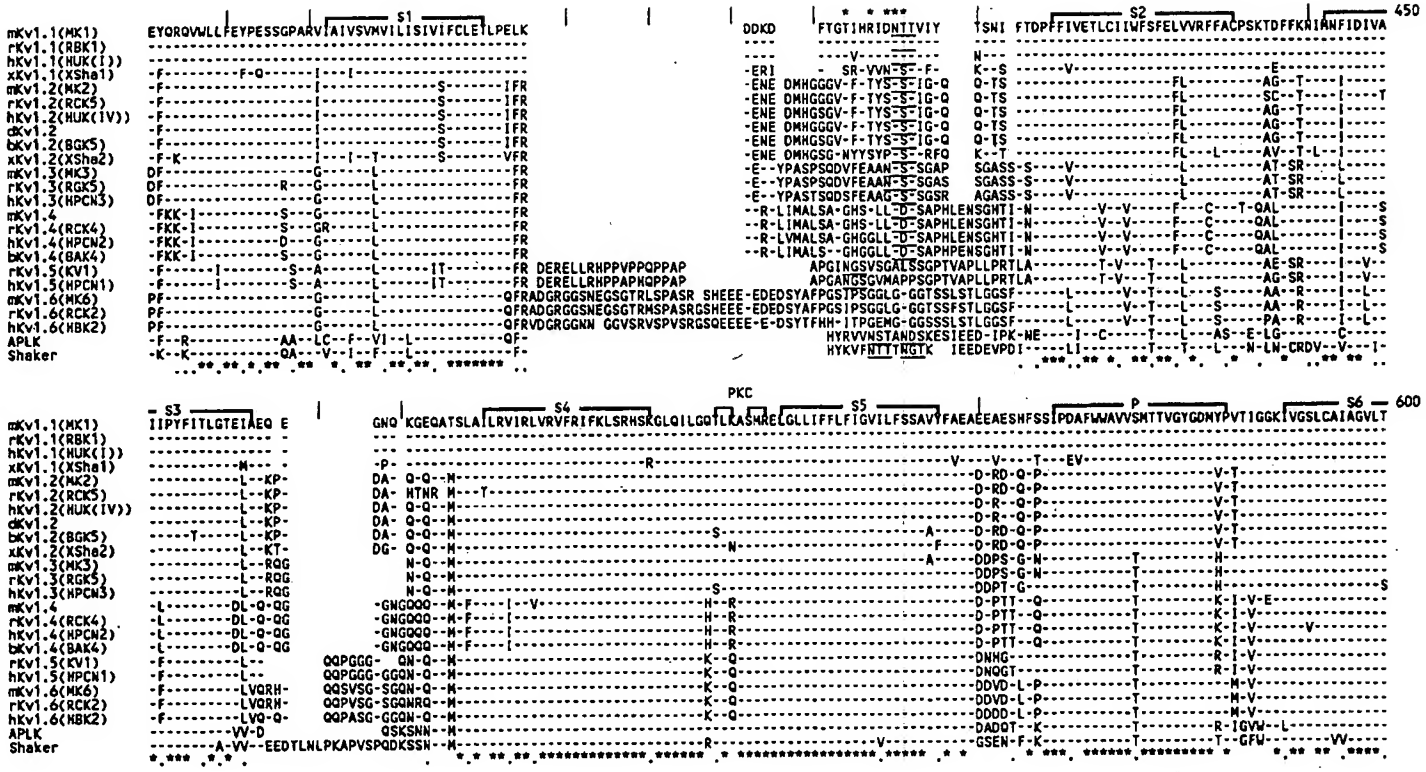

FIGURE 2(2).

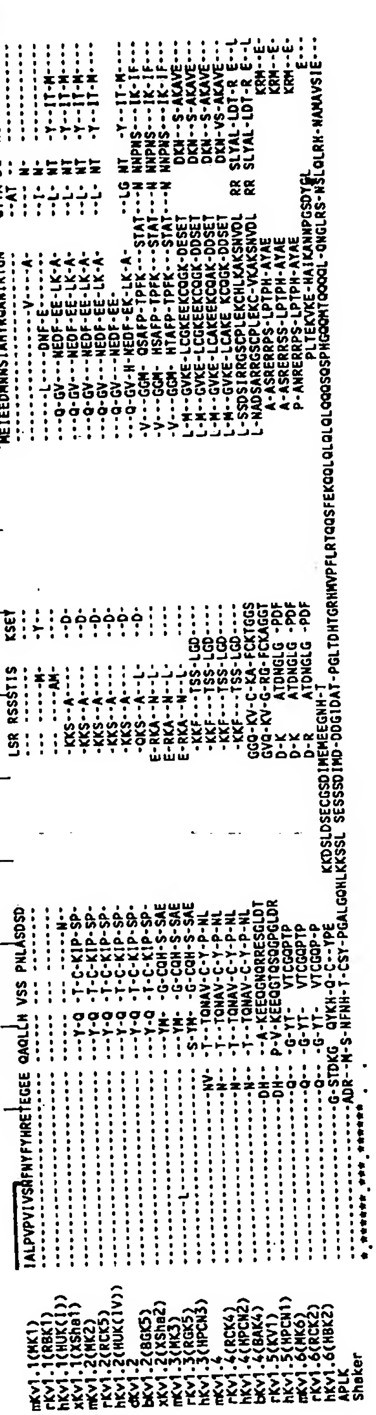

FIGURE 2(3).

their structure (since a region that is completely functionless would be expected to be progressively lost over evolutionary time). A clue to the nature of such functions may be provided by the fact that the Kv1.4 proteins have the longest N-terminal region of the mammalian *Shaker* subfamily, and also contain some strikingly repetitive sequences (e.g., runs of 11/13 alanines, 14/15 glutamines, as well as shorter runs of glycines, histidines and lysines); the presence of glutamine repeats in the N-terminal portion of *Shaker* has already been noted (Schwarz et al., 1988).

Simple repetitive sequences are widely dispersed throughout the genomes of eukaryotes, recent highly publicized examples including the trinucleotide repeats associated with mutations in the gene responsible for Huntington's disease (Huntington's Disease Collaborative Research Group, 1993), and the dinucleotide repeats reported to be associated with a predisposition to colorectal cancer (Aaltonen et al., 1993; Thibodeau et al., 1993). One mechanism by which simple repetitive sequences may be expanded is slipped-strand mispairing. It has been suggested that in nucleotide sequences constrained only by the requirement for their presence (and not for a particular structure) one might expect a progressive replacement by simple repeats (Levinson and Gutman, 1987), and the presence of such simple repeats in *Shaker* and *Shaker*-related genes might be the result of such a process. The required conditions could be fulfilled if, for example, the nonconserved region were required only to provide a suitable spacer arm ("chain") for the N-terminal inactivating "ball" known to be responsible for fast inactivation in *Shaker* (Jan and Jan, 1992). Kv1.4, in fact, is the only mammalian *Shaker* homologue that shows a *Shaker*-like rapid inactivation, and it also contains the longest N-terminal region as well as the most striking amino acid repeats within this family; these repeats begin immediately after a stretch of charged residues that are thought to form the fast-inactivation ball of this channel (indicated in Figure 2; see also Section 2.1.5). While other members of this family are delayed rectifiers and do not exhibit rapid "ball and chain" type inactivation, analogous "spacer" regions might be required for structures involved in subunit assembly or other functions, and the presence of simple amino acid repeats might be associated with such spacers.

2.1.1.1.2 Posttranslational Modification

Three potential sites of posttranslational modification are indicated in Figure 2 (as well as Figure 1). First, an N-glycosylation site (Marshall, 1972) is present in the extracellular loop between S1 and S2 in all the mammalian *Shaker*-related sequences shown in Figure 2 other than Kv1.6; the absence of a glycosylation site in this region is shared by both mouse and human Kv1.6. Two such sites are evident in the corresponding region of *Aplysia* and *Shaker* K+ channels. The presence of such a site in almost all of this subfamily of channels is all the more striking given the considerable divergence in both the sequence and length of the S1-S2 loop. Using an *in vitro* translation system, Shen et al. (1993) demonstrated glycosylation at this site in the *Aplysia* K+ channel, N-glycosylation of the *Shaker* channel has also been shown

(Rosenberg and East, 1992; Santacruz-Toloza et al., 1994); it has not yet been determined, however, whether this site is glycosylated in native channels. Other N-glycosylation consensus sequences exist in these and other channels, including several in the C-terminal region, but as these are thought to be located intracellularly they are unlikely to be utilized and we do not indicate them in any of our alignments.

Second, all *Shaker*-related proteins have a potential tyrosine kinase site in the N-terminal region (RPSFDAILY), indicated in Figure 2; while its conservation suggests it is functional, this site has not been experimentally shown to be utilized, and deletion of this region from the mKv1.3 channel had no apparent affect on channel function (Aiyar et al., 1993a). Third, all of the *Shaker*-subfamily proteins show one or two protein kinase C (PKC) consensus sites (Ser/Thr-X-Arg/Lys) in the cytoplasmic loop between S4 and S5 (Kemp and Pearson, 1990), a region that has been modeled to form part of the internal surface of the ion conduction pathway (Isacoff et al., 1991; Jan and Jan, 1992; Durell and Guy, 1992). In fact, a PKC site in this region is present in *all* known voltage-gated K^+ channels (further discussed below), and there is evidence that phosphorylation of this site can affect channel function. Phorbol esters, which activate PKC, suppress Kv1.3 currents in oocytes and in Jurkat T cells, probably by phosphorylating these sites, and the effect is reversibly inhibited by PKC inhibitors (Attali et al., 1992a; Payet and Dupuis, 1992; Aiyar et al., 1993b). Rat Kv1.3, translated *in vitro,* has been reported to be phosphorylated by PKC, presumably at one or both of these sites (Cai and Douglass, 1993). Similar results have been reported for the rKv3.1 (Critz et al., 1993), hKv1.4 (Fahrig et al., 1992), rKv4.2 (Blair et al., 1992), and *Shaker* channels (Moran et al., 1991), and removal of both PKC sites by site-directed mutation (K → D and R → D) in the *Shaker* protein produced nonfunctional channels (Isacoff et al., 1992).

2.1.1.1.3 Species Conservation

The mammalian *Shaker*-related genes generally show a high level of interspecies conservation. For example, comparison of the Kv1.2 protein sequences of human, rat, mouse, dog, and cattle reveals greater than 98% identity over the entire protein (Figure 3A); even the more distantly related *Xenopus* sequence is greater than 91% identical to the mouse. This is not universally true, however, and one exception is illustrated in Figure 3B, which shows that rat and human Kv1.5 are only 87% identical overall (ignoring one 11-residue size difference), and less than 64% identical in the amino-terminal 100 residues. The more typical Kv1.2, on the other hand, shows 99.4% overall identity between its human and rodent homologues. These apparent differences in rates of divergence are not the consequence of sequencing artifacts, since the nucleotide sequences of the five human Kv1.5 cDNAs that have been independently isolated are identical in this region (Ramaswami et al., 1990; Philipson et al., 1991; Tamkun et al., 1991; Curran et al., 1992; Fedida et al., 1993), as are the sequences of three independent rat Kv1.5 clones (Swanson et al., 1990).

FIGURE 3. (A) Amino acid sequence alignment of rat, mouse, human, dog, bovine (partial sequence), and frog homologues of Kv1.2, illustrating their high degree of similarity. Putative functional regions are indicated as in Figure 2. (B) Amino acid sequence alignment of rat and human homologues of Kv1.5, showing their unusually extensive sequence divergence in the N-terminal region. Putative functional regions are indicated as in Figure 2.

FIGURE 3B.

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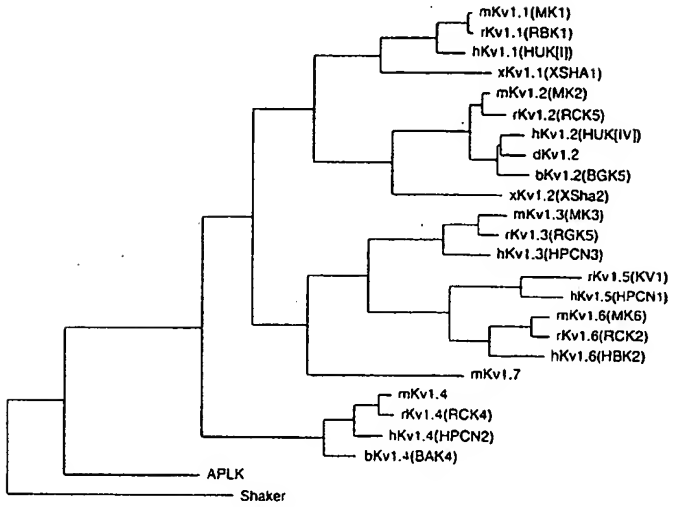

FIGURE 4. Proposed phylogenetic tree of *Shaker*-related K⁺ channel genes, based on parsimony analysis of nucleotide sequence alignments using the program PAUP (Swofford, 1993). The placement of mKv1.7 is poorly supported (see text).

Two additional *Shaker*-related mammalian genes are known to exist, but sequences for these genes either exist only as short published fragments [mouse and rat Kv1.7 (Betsholtz et al., 1990)] or have not yet been published [mouse Kv1.8 (B. Tempel, personal communication); mouse Kv1.7, K. Kalman et al., unpublished data].

2.1.1.1.4 *Shaker*-Subfamily Phylogenetic Tree

A proposed phylogeny for the *Shaker*-related genes is shown in Figure 4. While our earlier analyses placed the *Xenopus* Xsha2 gene outside the mammalian group (Strong et al., 1993), the current larger dataset places each of the two *Xenopus* genes within one of the mammalian groups of homologues, Xsha1 with Kv1.1 and Xsha2 with Kv1.2. This larger dataset also positions the Kv1.2 group differently than previously, making it a sister group of the Kv1.1 cluster. The remaining features of this tree are the same as we have already proposed (Strong et al., 1993), including our inability to determine the position the mouse Kv1.7 sequence in this tree with any degree of confidence. It should be noted that the terminal patterns of branching in the Kv1.2 and Kv1.4 clusters, the two that include bovine homologues as well as rodent and human, are inconsistent with each other. Neither of these patterns is compellingly supported by bootstrap analysis, and this inconsistency reflects the current uncertainty regarding the true phylogenetic relationships between many mammalian orders (Novacek, 1992; Graur, 1993).

If this phylogeny is correct, then the duplications that gave rise to the mammalian *Shaker*-related subfamily of genes must have occurred before the

divergence of mammals and amphibians, some 350 million years ago (MYA; for discussion of times of divergence see Nei, 1987). Reptiles and birds, which diverged from mammals more recently (about 300 MYA), would therefore be expected to also show the presence of multiple members of the known mammalian homologues, and the nomenclature proposed for mammalian K^+ channel genes (Chandy et al., 1991; Gutman and Chandy, 1993) should also be applicable to these other vertebrates when cloned genes become available. The placement of the *Aplysia* sequence outside the mammalian group implies that the vertebrate duplications occurred after the divergence of molluscs as well as insects from the vertebrate lineage (~600 MYA). Whether these duplications predate the divergence of older vertebrate lineages, i.e., fish and cyclostomes (400–450 MYA), remains to be determined.

2.1.1.2 Genomic Organization

The *Shaker* gene in *Drosophila* contains multiple exons spread over 120 kb and can be alternatively spliced to generate at least five distinct functional transcripts (Pongs et al., 1988; Schwarz et al., 1988). The various resulting *Drosophila Shaker* channels inactivate with different time courses (Timpe et al., 1988; Stocker et al., 1990; Iverson et al., 1990; Wittka et al., 1991); ShB1, for example, inactivates much more rapidly than ShA1, although it recovers equally rapidly from inactivation (Stocker et al., 1990; Iverson et al., 1988; Witta et al., 1991).

In dramatic contrast, the coding regions of all but one of the known mammalian *Shaker* homologues, Kv1.1-Kv1.6, and Kv1.8, are uninterrupted in the genome (Chandy et al., 1990b; Swanson et al., 1990; Douglass et al., 1990; Wymore et al., 1994; B. Tempel, personal communication). The Kv1.7 gene is the only known exception, having at least one intron in its coding sequence in the loop linking S1–S2 (Chandy et al., 1990a; K. Kalman et al., unpublished data). While the existence of intronless coding regions would seem to preclude the generation of diverse channel proteins through alternate exon utilization, different forms of protein could nevertheless be generated by alternative use of undiscovered splice sites, either sites defining new introns, or potential sites within known coding regions (see cautions regarding sequence interpretation in footnote). The *Xenopus* Kv1.1 and Kv1.2 genes also lack introns in their coding regions (Ribera, 1990; Ribera and Nguyen, 1993) suggesting that this genomic organization may be common to all vertebrates. The evolutionary significance of this difference in the structure of fly and vertebrate channel genes and the mechanisms by which this family of intronless genes have evolved remain obscure.

2.1.1.2.1 5′-Noncoding Introns in Kv1.1 and Kv1.2; Posttranscriptional Regulation?

Kv1.1–Kv1.3 genes express several large transcripts (8–9.5 kb) along with several smaller ones (Tempel et al., 1988; Stuhmer et al., 1989; McKinnon,

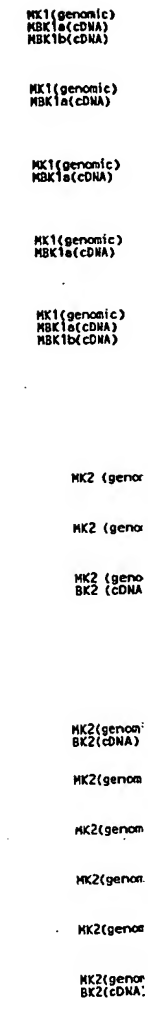

FIGURE 5.
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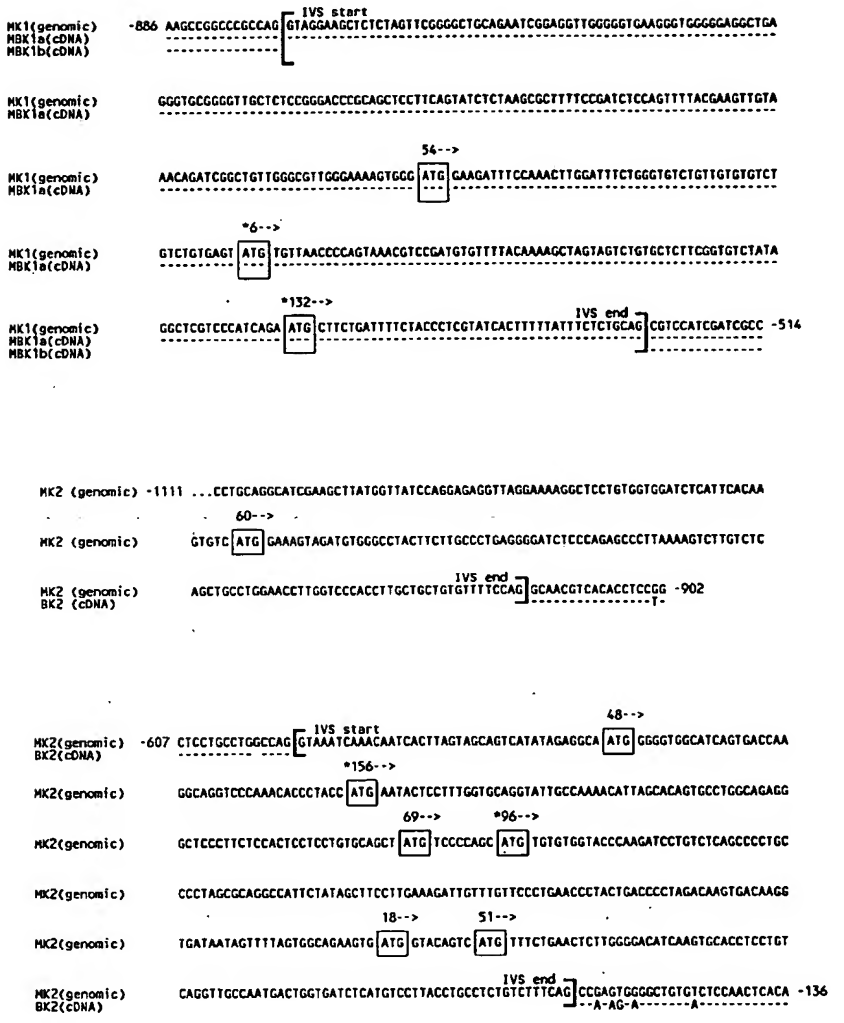


FIGURE 5. (A) Nucleotide sequence alignment of a portion of the genomic 5′ untranslated sequence (5′-UTS) of mouse mKv1.1 (from position −886 to −514 relative to the beginning of the MK1 protein-coding region), with two distinct mKv1.1 cDNAs (MBK1a and b). The borders of an intervening sequence present both in the genomic sequence and in MBK1a are indicated by brackets. Three potential translation start sites (PNNATG, where P indicates either A or G, and N indicates any nucleotide) are boxed, and the lengths of their downstream open reading frames are indicated above. Two sites that display the preferred initiation sequence, ANNATG, are indicated by asterisks. (B) Alignment of a portion of the genomic 5′-UTS of mouse Kv1.2 (from position −1111 to −902) with that of a rat Kv1.2 cDNA. The 3′ end of a putative intervening sequence is indicated by a bracket, and a potential translational start site is indicated as in A. (C) Alignment of another region of the genomic 5′-UTS of mouse Kv1.2 (from position −607 to −136) with that of a rat Kv1.2 cDNA, showing the presence of an intron whose ends are indicated by brackets. Six potential translation start sites are indicated as in A and B.

1989; Douglass et al., 1990; Spencer et al., 1993). In the case of mKv1.1, alternative splicing of a short intron within its 5′-NCR has been shown to yield both spliced and unspliced polyadenylated RNA (Tempel et al., 1988). As seen in Figure 5A, this 5′ intron (position –886 to –514) contains three potential initiation codons (consensus sequence PNNATG, where P indicates either A or G, and N is any nucleotide), each generating an open reading frame whose length is indicated above the ATG codon; two of these (indicated by asterisks) show the preferred consensus sequence ANNATG. According to the scanning model for translation initiation (Kozak, 1991), these upstream ATGs could inhibit translation of the Kv1.1 protein by delaying the arrival of scanning ribosomes at the authentic start site; Kv1.1 protein might therefore be inefficiently produced from transcripts which contain this intron.

Comparison of the mKv1.2 genomic sequence with that of rKv1.2 cDNA also reveals the presence of at least two introns in the 5′-NCR (Figure 5B,C). We have identified only the splice acceptor site of the upstream intron, at position –920 (Figure 5B), and this putative intron contains at least one ATG initiation codon. The downstream intron (–607 to –136) contains 6 possible translational start sites producing open reading frames ranging from 18 to 156 bp (Figure 5C), two of which match the preferred Kozak consensus sequence.

The existence of 5′ introns containing potential initiation codons raises the intriguing possibility of post-transcriptional regulation of K^+ channel expression by alternative splicing in the 5′-UTS. Kozak (1991) has reviewed examples of messenger RNAs of differing translational efficiency being produced by alternative sites of transcription initiation, and of the demonstrated deleterious effects of upstream ATG codons on translational efficiency. Her cautionary notes regarding the difficulty of unambiguously identifying the structure of functional mRNAs are particularly relevant to our discussion; in the case of Kv1.1, for example, neither end of the transcription unit has been identified, and the intron-containing form of mRNA has not been demonstrated to be functional. Nevertheless, mRNAs containing this intron appear to constitute a substantial minority of Kv1.1 messenger RNA in mouse brain (Tempel et al., 1988), and alternative splicing of such introns could potentially play a role in both the tissue specificity of expression of Kv proteins and in their up- and down-regulation.

2.1.1.2.2 Mapping Kv1.4 and Kv1.5 mRNAs

The transcripts of Kv1.4 and Kv1.5 are generally shorter (2.4–4.5 kb) than those of Kv1.1–Kv1.3 (Tamkun et al., 1991; Swanson et al., 1990; Philipson et al., 1990). A schematic diagram of the mKv1.4 gene and its transcripts is shown in Figure 6. mKv1.4 appears to have a single, large intron (3.4 kb) in its 5′-NCR (Wymore et al., 1994). Like most other members of the *Shaker*-related family, however, its protein coding sequence, as well as an additional 2 kb of 5′- and 3′-UTS, is contiguous in the genome. Although neither the transcription initiation site nor the 3′ polyadenylation site of this mRNA has been definitively identified, they are unlikely to be far from the limits indicated in the figure, defined by the 5′ end of hPCN2 and the 3′ end of RHK1.

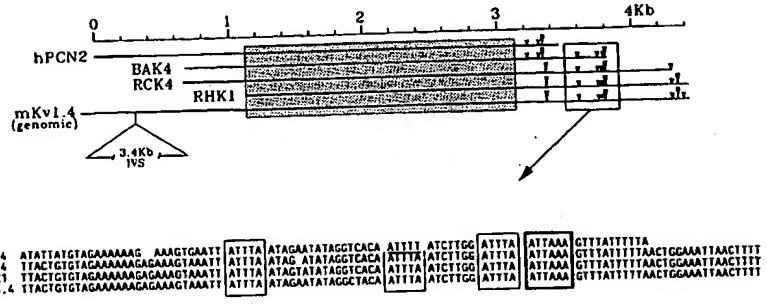


FIGURE 6. Schematic diagram (above) of Kv1.4 transcripts from human (hPCN2), cattle (BAK4), rat (RCK4 and RHK1), and genomic DNA from mouse Kv1.4. The shaded box encloses the protein coding region. Within the 3′-UTS are indicated the positions of potential polyadenylation signals (tall arrowheads) and ATTTA motifs (short arrowheads) implicated in destabilization of mRNA (see text). The aligned nucleotide sequences for the boxed region in the 3′-UTS are shown below, with the polyadenylation signals and ATTA sequences indicated. The ruler above indicates nucleotides (in kb) beginning with the 5′ end of the hPCN2 transcript.

A total of more than 4.4 kb of the Kv1.4 transcript is thus accounted for in the mouse genomic sequence, leaving no more than about 100 bp possibly remaining at both ends of the longest message.

Unusual features of the 3′-UTS of Kv1.4 suggest the possibility of this region playing a role in posttranscriptional regulation of expression. The rat, mouse, and bovine Kv1.4 genes have at least three polyadenylation recognition signals in the 3′-NCR, which are indicated in Figure 6. Differential utilization of these signals may be partially responsible for the generation of the three Kv1.4 mRNAs (2.4, 3.5, and 4.5 kb), although omission of a large portion of the 5′-NCR (through an alternative transcriptional start site, or splicing of an unidentified intron) would also need to be invoked to account for a functional message as small as 2.4 kb. Many conserved ATTTA sequences are also present in the 3′-NCRs of the rat, mouse, and bovine Kv1.4 genes (also shown in Figure 6), motifs that have been reported to reduce mRNA stability when present in the 3′-NCRs of cytokine genes (Shaw and Kamen, 1986). If the presence of these sequences has a destabilizing effect on Kv1.4 transcripts, then one might expect that the shortest Kv1.4 mRNAs, those containing the fewest ATTTA sequences, would be more stable than longer transcripts and may therefore be the most efficiently translated. Such AT-rich sequences have also been reported to interfere with translation, independently of their effects on message stability (Kruys et al., 1989). It is worth noting that the shortest Kv1.4 transcripts, 2.4 kb in length, can have no more than 400 nt of total UTS; therefore, if they have intact protein coding regions, they must lack *all* the ATTTA motifs indicated in Figure 6.

The 5′-NCR of rKv1.5 as well as its protein coding region appear to be intronless, and its transcription start site has been reported to be located 774

bp upstream of the initiating ATG (Mori et al., 1993). A cAMP response element located in the 5′-NCR at position +636 (relative to the transcription start site) confers cAMP responsiveness and binds CREB and CREM DNA-binding proteins (Mori et al., 1993). Recently, Shelton et al. (1993) have used the β-globin locus-control region (LCR) to direct transcription from the hKv1.5 promoter region, located just upstream of the coding region, resulting in the expression of hKv1.5 currents in mouse erythroleukemic cells.

2.1.1.2.3 Chromosomal Locations

The Kv1.1, Kv1.5, and Kv1.6 genes have been mapped to mouse chromosome 6 (Lock et al., 1994) near the *ophisthotonus* and *deaf waddler* loci, and have been recovered together on a single yeast artificial chromosome (YAC) about 275 kb in length (Migeon et al., 1992). No recombination was detected between these three genes in 113 backcrosses studied, confirming their close proximity (Lock et al., 1994). Human Kv1.1 has been localized to human 12p13, a region homologous to mouse chromosome 6 (Grissmer et al., 1992; Wymore et al., 1994); hKv1.5 and hKv1.6 would therefore also be expected to map to this region. In fact, hKv1.5 has been mapped to human 12p13 (Curran et al., 1992; Attali et al., 1993). Mutations in hKv1.1, 1.5, or 1.6 might therefore result in human diseases linked to human 12p13, possibly resembling *ophisthotonus* or *deaf waddler,* although none have yet been described. Trisomy of the 12p region is associated with an epileptiform disorder (Guerrini et al., 1990), and it is tempting to implicate one of these three K^+ channel genes in this disorder. One other *Shaker*-subfamily gene has been reported to be on human chromosome 12, namely Kv1.2 (Grissmer et al., 1990); however, its mouse homologue maps to mouse chromosome 3, which is homologous to human 1p (Lock et al., 1994), and this conflict needs to be resolved.

Human Kv1.3 has been mapped to human chromosome 1p13/1p21 (Table 1; Attali et al., 1992b); its mouse homologue mKv1.3 as well as mKv1.8 have also been mapped to mouse chromosome 3 (Lock et al., 1994). Kv1.7 lies on human 19q13.3 (K. Kalman et al., unpublished), and on mouse chromosome 7 in close proximity to mKv3.3 (K. Kalman et al., unpublished). The recessive mutant mouse gene *quivering* is also located in this region.

The defective gene for some forms of the long QT (LQT) syndrome, an autosomal dominant cardiac disease, lies within 10 cM of H-*ras* at human chromosome 11p15.5 (Keating et al., 1991; Wymore et al., 1994). The QT electrocardiogram interval, which is abnormally long in this disease, is a measure of cardiac repolarization. Since K^+ channel opening during the action potential is responsible for cardiac repolarization, a defect in a cardiac Kv gene such as Kv1.4 could lead to the prolongation of the QT interval, and consequently to the LQT syndrome. In the mouse, Kv1.4 is located close to the gene encoding FSHB (follicle stimulating hormone B) on chromosome 2 (Wymore et al., 1994), and it occupies a homologous position at human chromosome 11p14.3, approximately 25 Mb from H-*ras* (Gessler et al., 1993; Wymore et al., 1994). Thus, Kv1.4 appears to be too far from H-*ras* to be considered a candidate gene at least for this form of the LQT syndrome. However, the *anx*

gene that causes anorexia, body tremors, and uncoordinated gait in mice also maps to mouse chromosome 2 in the same region as mKv1.4 (Maltais et al., 1984).

2.1.2 *Shab-, Shaw-,* and *Shal*-Subfamily Genes

2.1.2.1 *Shab*-Subfamily

Only two mammalian *Shab*-related isoforms are known, Kv2.1 and Kv2.2, represented by five cDNA sequences, one from mouse and two each from rat and human (Table 1). An amino acid sequence alignment of the rat homologues of Kv2.1 and Kv2.2, together with the fly *Shab* gene, is shown in Figure 7. Some of the features discussed for the *Shaker* alignment hold for this comparison as well, namely the striking conservation of the hydrophobic core of the proteins and the presence of a PKC site in the S4–S5 intracellular loop. However, the S1/S2 loop, which in the *Shaker* channels is highly variable in length and in sequence, is represented by a short conserved segment in the *Shab*-related proteins; while the *Drosophila Shab* protein has an N-glycosylation site in this segment (as do most *Shaker*-related channels), this site is absent from both of its mammalian homologues. The S3–S4 extracellular loop is also shorter and more highly conserved in *Shab*-subfamily genes, and this loop contains an N-glycosylation site in all three proteins. A region of 200 N-terminal residues adjacent to S1 is very highly conserved between all three proteins, while the *Shab* protein has about 240 additional residues at its N-terminus, marked by the presence of some strikingly repetitive segments (e.g., poly-Glu, poly-GlyAla). On the other hand, the long C-terminal region beginning about 100 residues past the end of S6 (the longest among all mammalian Kv proteins) shows very little sequence conservation, a feature similar to the *Shaker* comparisons. It remains possible, however, that alternate splicing at the 3′ end of the coding regions of the *Shab* genes may contribute to this diversity, as it does for *Shaw*- and *Shal*-related channels (see below), since nothing is yet known about the intron/exon structure of the *Shab*-subfamily genes. Kv2.1 and Kv2.2 are located on mouse chromosomes 2 (some distance from mKv1.4) and 1, respectively (Lock et al., 1994).

2.1.2.2 *Shaw*-Subfamily

2.1.2.2.1 Amino Acid Sequence Alignment

Four mammalian *Shaw* homologues have been described (Kv3.1–Kv3.4; Table 1), and an alignment of four representative protein sequences from rats together with the *Shaw* sequence is shown in Figure 8. The hydrophobic core is highly conserved, as well as a ~70-residue region within the N-terminal region, but here separated from the hydrophobic core by a highly variable segment of 50–70 residues. Unlike the mammalian *Shaker* homologues that have seven positive charges as part of the repeated motif in S4 (a positively charged residue at every third position), the *Shaw* channel has only four positive charges in the region, which has been aligned under the mammalian S4, lacking the first two arginines through one deletion and one substitution.

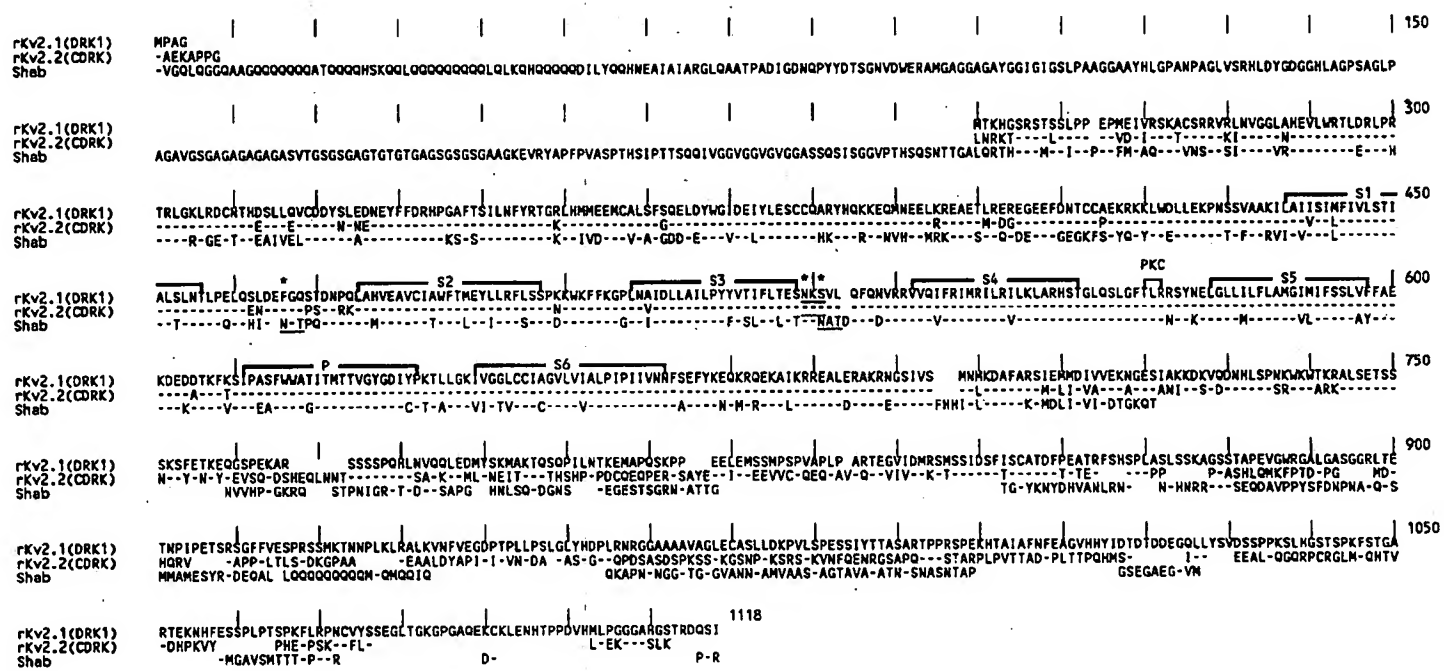
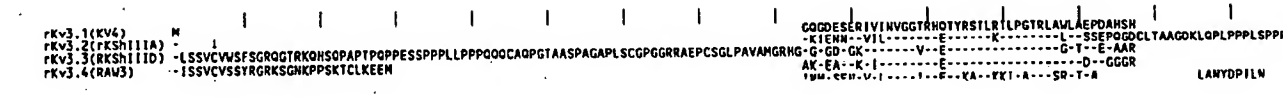


FIGURE 7. *Shab*-family amino acid sequence alignment, including the rat homologs of Kv2.1 and Kv2.2 and the *Drosophila Shab* protein. Putative functional regions are indicated as in Figure 2.



protein. Putative functional regions are indicated as in Figure 2.

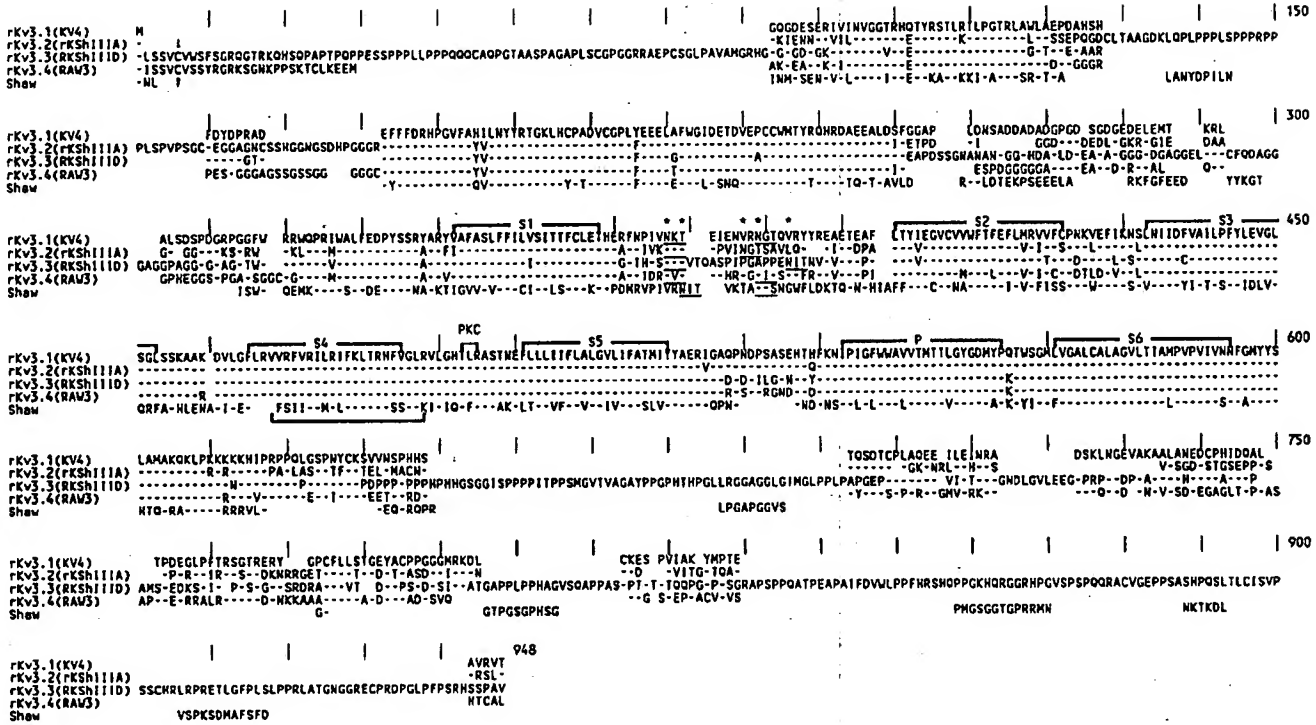


FIGURE 8. *Shaw*-family amino acid sequence alignment, including the rat homologs of Kv3.1–Kv3.4 and the *Drosophila Shaw* protein. Putative functional regions are indicated as in Figure 2. The proposed S4 region of the *Shaw* protein is indicated by a bracket below, in consideration of the size difference evident between this protein and its mammalian homologues (see text). Arrows mark the position of a cysteine residue at position 6 of rKv3.3 and rKv3.4, suggested to correspond to the cysteine involved in fast inactivation in Kv1.4 (see Figure 2, and text).

However, an additional arginine is present six residues downstream, which is in phase with the last arginine in the repeated motif. Given the three-residue deletion in *Shaw* relative to its mammalian homologues, its S4 region should probably be extended to this arginine (as indicated by the bracket below the alignment), thus bringing to five the total number of positive charges in S4, which might contribute to voltage sensing (discussed further below).

2.1.2.2.2 Posttranslational Modification

Two N-glycosylation sites are present in the poorly conserved S1/S2 loop of all of these *Shaw*-related proteins, and the universal PKC site is present between S4 and S5. There is a highly variable C-terminal region, although alternate mRNA splicing (discussed below) is known to contribute to this diversity. Regions containing short amino acid repeats are evident, particularly in the N-terminal region of Kv3.2 and in the C-terminal insertion just outside the conserved core in Kv3.3.

2.1.2.2.3 Introns and Alternative Splicing

The transcripts of all four *Shaw* genes are heterogeneous and include large forms, ranging from 4.5–13 kb, and none has yet been mapped. While complete genomic sequence is not available for any *Shaw*-related protein, one intron is known to be present at the 5′ margin of the S1 transmembrane segment in Kv3.1, 3.3, and 3.4 (Ghanshani et al., 1992; Luneau et al., 1991a,b). In addition, alternative splicing is known to contribute to the diversity of the C-termini of Kv3.1 and Kv3.2; the Kv3.1b channel differs from its alternately spliced counterpart, Kv3.1a, in that the last 10 amino acids of Kv3.1a are replaced by an unrelated sequence of 84 amino acids in Kv3.1b. In the case of Kv3.2, alternative splicing at their 3′ ends can yield four distinct Kv3.2 mRNAs, as shown schematically in Figure 9. Two of these transcripts (RAW1/rKShIIIA2 and rKShIIIA) share a common 3′ segment, indicated by cross-hatching; this shared region does not contribute to the translated product of the RAW1/rKShIIIA2 transcript, however, due to the presence of a termination codon (indicated by a heavy bar) in the shorter exon, which is absent from the rKShIIIA transcript (Luneau et al., 1991b; Rettig et al., 1992). While these two exons are shown as being separated by an intervening sequence, the existence of such an intron is unproven, since the genomic sequence is not known; the two exons might be contiguous in the genome, as they are in the RAW1/rKShIIIA2 transcript, and the rKShIIIA transcript would then result from the use of a splice acceptor site within this exon. The same caution holds true for many other Kv genes for which multiple transcripts are known but no genomic sequence is available.

2.1.2.2.4 *Shaw*-Subfamily Phylogenetic Tree

Figure 10 shows a proposed phylogenetic tree of all known *Shaw*-related genes, resolving the ambiguities in our earlier analyses, which resulted from a paucity of data (Strong et al., 1993). This phylogeny implies that three separate gene duplications of a *Shaw*-related ancestor gave rise sequentially to the four known mammalian *Shaw* homologues. No nonmammalian *Shaw*-

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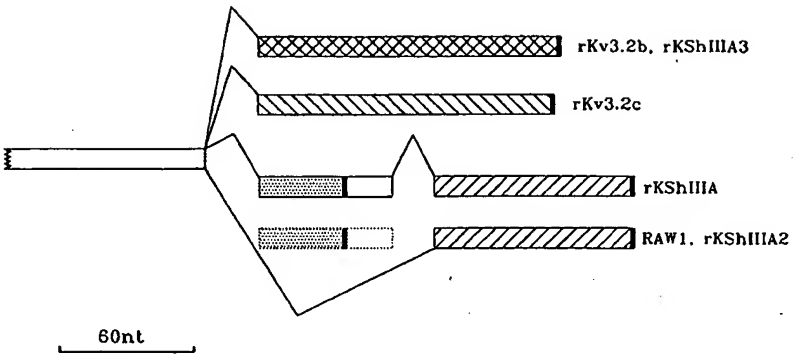

FIGURE 9. Four alternatively spliced variants at the 3′ end of rat Kv3.2 mRNAs, showing the hypothetical splicing patterns. Four distinct exon-containing regions are indicated by different fill patterns, and the positions of translation termination codons are indicated by solid vertical bars. The scale bar indicates a length of 60 nucleotides.

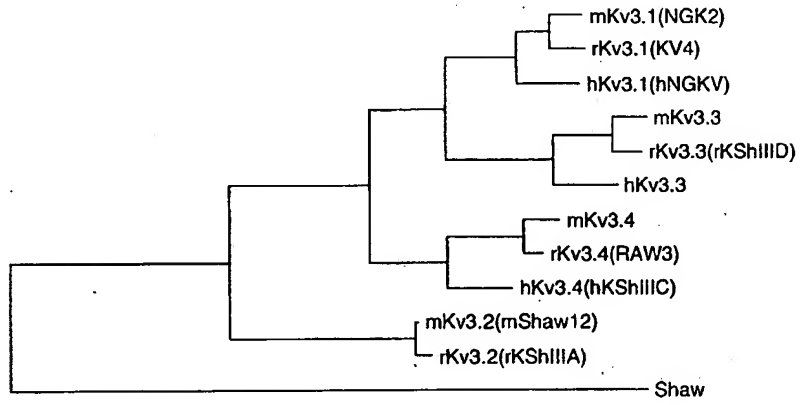

FIGURE 10. Proposed phylogenetic tree of *Shaw*-related K⁺ channel genes, based on parsimony analysis of nucleotide sequence alignments using the program PAUP (Swofford, 1993).

related sequences are yet available to determine at what period in the vertebrate radiation these duplications occurred.

2.1.2.2.5 Chromosomal Locations

The four *Shaw*-related genes (Kv3.1, Kv3.2, Kv3.3, Kv3.4) have been reported to be located on human chromosomes 11, 12/19, 19, and 1, respectively (see references in Table 1). Kv3.1 has been sublocalized to human chromosome 11p14.3–15.2 (Reid et al., 1993; Wymore et al., 1994); it appears to be about >25 Mb away from H-*ras*, and therefore is unlikely to be a candidate gene for the H-*ras*-linked form of the LQT syndrome (Wymore

et al., 1994). In the mouse, Kv3.1 is within 1 cM of myoD1 on chromosome 7 (Wymore et al., 1994), a region that is homologous to human 11p15.1/15.2 (Reid et al., 1993; Wymore et al., 1994). While Kv3.2 maps to mouse chromosome 10 in a region homologous to human 12q (Lock et al., 1994), conflicting reports have placed it on human 12 (Curran et al., 1992; McPherson et al., 1991) and on 19q13.3-13.4 (Haas et al., 1993). Kv3.3 lies close to Kv1.7 both in the mouse (on chromosome 7) and in humans (on human 19q13.3; K. Kalman et al., unpublished data). Kv3.4 has been mapped to mouse chromosome 3 and the homologous region of human 1p21 (Rudy et al., 1991a; Ghanshani et al., 1992; B. Tempel, personal communication).

2.1.2.3 *Shal*-Subfamily

An alignment of proteins representing two mammalian isoforms of *Shal*-related channels is shown in Figure 11, together with that of the *Drosophila Shal* protein; one additional *Shal*-related mammalian gene, Kv4.3, is known to exist, but its sequence has not yet been published (Rudy et al., 1991b). The hydrophobic core is highly conserved, and the universal PKC site is present between S4 and S5. A potential tyrosine kinase site is present in the N-terminal region of Kv4.2, but not Kv4.1, and the only potential external N-glycosylation site is in the extracellular loop linking S5 to the P-region of Kv4.1 and *Shal* (but not Kv4.2). Interestingly, sodium channel proteins are known to be heavily N-glycosylated, at a position thought to be between S5 and the pore region (Miller et al., 1983; James and Agnew, 1987; Ukomadu et al., 1992). The S4 segment of the *Shal*-subfamily genes contains only six positive charges that are part of the repeated motif, in contrast to the 7 positive charges present in *Shaker*-subfamily genes. Like the *Shab* subfamily (but unlike the *Shaker* and *Shaw* families), the N-terminal region of *Shal* subfamily genes is almost perfectly conserved in its length, and substantially conserved in its sequence. While the C-terminal regions show extensive sequence divergence, nothing is known of possible alternative 3′ splicing within these genes. Kv4.1 is located on the mouse X chromosome (Lock et al., 1994).

2.1.3 The Extended Voltage-Gated K^+ Channel Gene Family: Amino Acid Sequence Comparisons

Figure 12 shows an alignment of the amino acid sequences of 16 mammalian K^+ channel genes, one representative of every known mammalian Kv gene, together with the four known fly homologues. There is clearly substantial sequence conservation in a large portion of the hydrophobic core of these proteins, as well as a more limited region in the N-terminal portion, about 100 residues away from S1; these relatively conserved regions are indicated by brackets below the alignment. Within these regions, comprising some 262 residues altogether, 44 positions show complete identity among all 20 sequences, while at an additional 65 positions only conservative substitutions

FIGURE 11. *Shal*-family amino acid sequence alignment, including the rat homologs of Kv4.1 and Kv4.2 and the *Drosophila* *Shal* protein. Putative functional regions are indicated as in Figure 2. The "RKR" sequence at position 36 of rKv4.2, known to be important in fast inactivation (see text), is underlined.

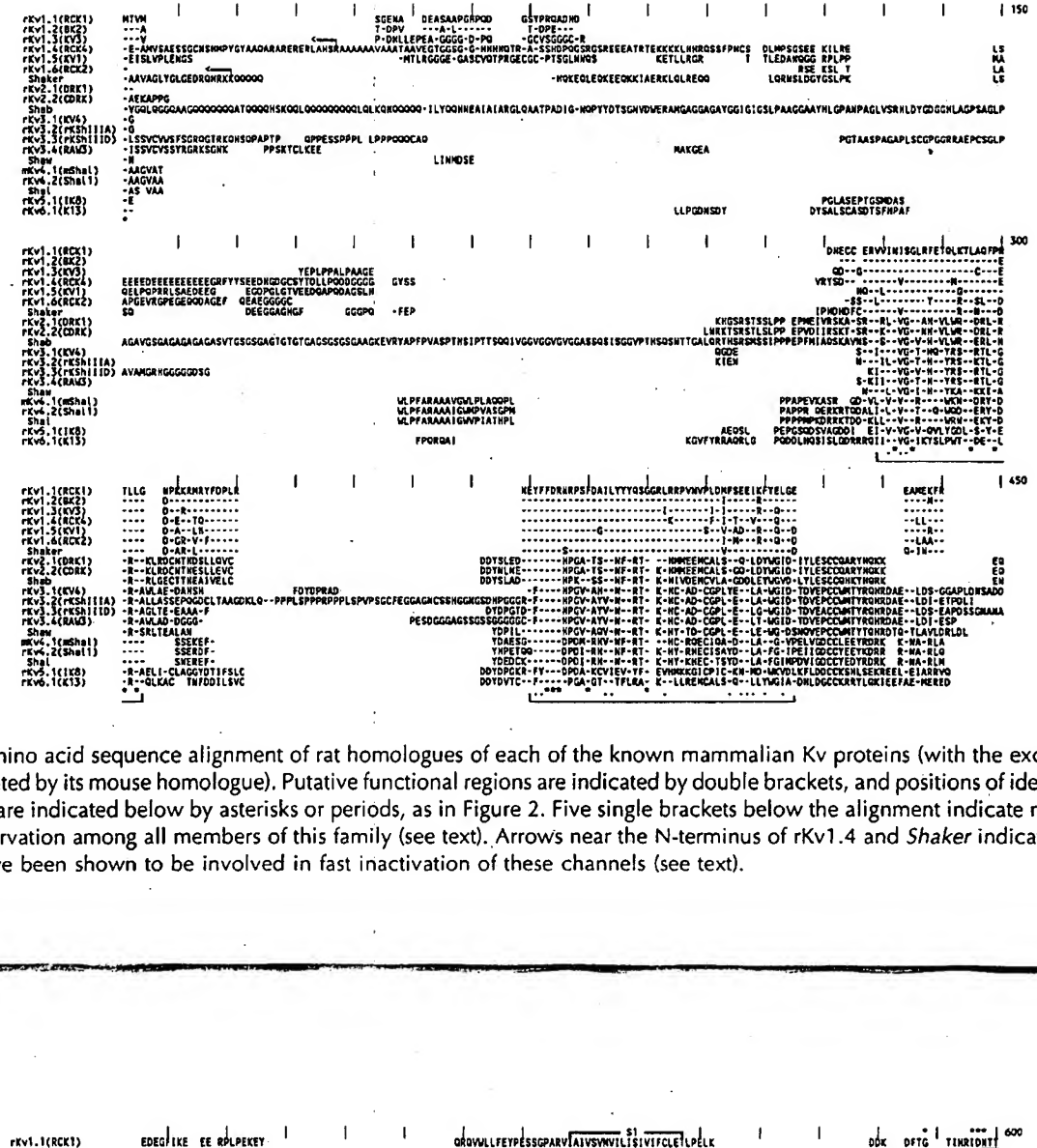

FIGURE 12. Amino acid sequence alignment of rat homologues of each of the known mammalian Kv proteins (with the exception of Kv4.1, which is represented by its mouse homologue). Putative functional regions are indicated by double brackets, and positions of identity or similarity in all sequences are indicated below by asterisks or periods, as in Figure 2. Five single brackets below the alignment indicate regions that show substantial conservation among all members of this family (see text). Arrows near the N-terminus of rKv1.4 and *Shaker* indicate the margins of peptides that have been shown to be involved in fast inactivation of these channels (see text).

which is represented by its mouse homologue). Putative functional regions are indicated by double brackets, and positions of identity or similarity in all sequences are indicated below by asterisks or periods, as in Figure 2. Five single brackets below the alignment indicate regions that show substantial conservation among all members of this family (see text). Arrows near the N-terminus of rKv1.4 and *Shaker* indicate the margins of peptides that have been shown to be involved in fast inactivation of these channels (see text).

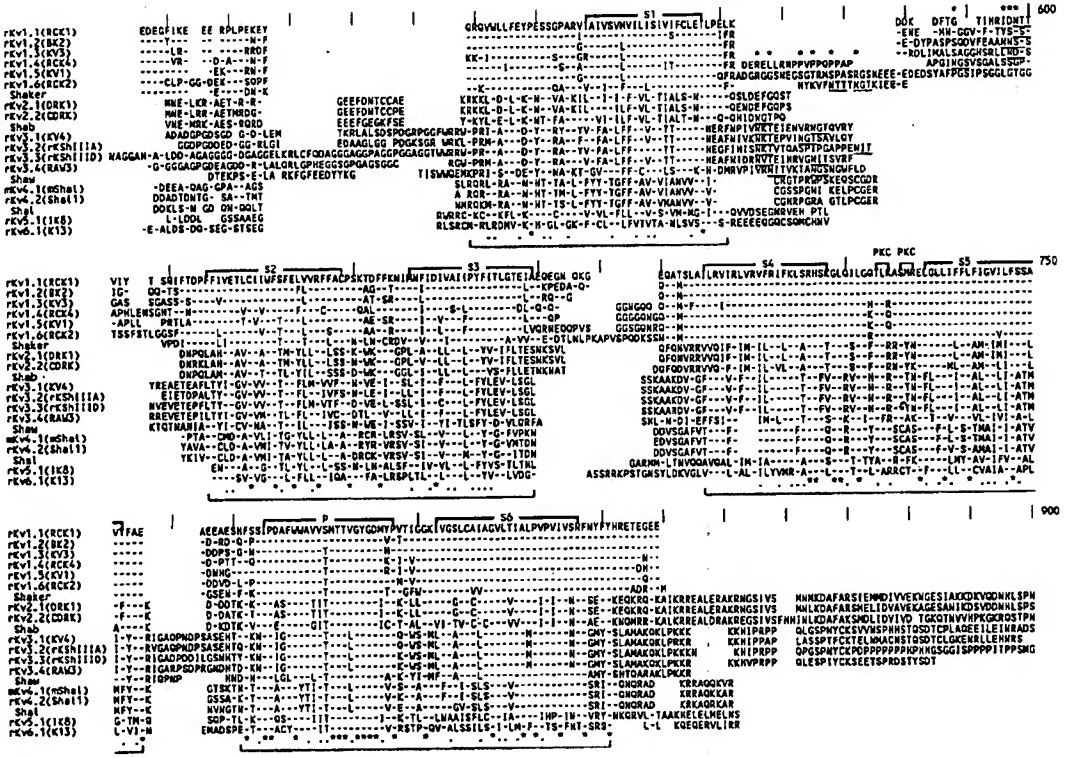

FIGURE 12(2).

FIGURE 12(3).

are seen. In current models of K^+ channels, the six membrane-spanning domains define the overall architecture of the channels and determine its gating properties, and the P-region participates in forming the ion-conducting pore (Durell and Guy, 1992); substantial conservation of these sequences is therefore not difficult to understand.

2.1.3.1 Amino and Carboxy-Terminal Regions

While size differences within the hydrophobic core of Kv channels is limited to the regions between the putative membrane-spanning segments, there is considerable diversity in the length of their C- and N-terminal regions, as illustrated schematically in Figure 13. The *Shaker*-related channels all have longer N-terminal regions than C-terminal; the *Shab*-related proteins are the longest overall, and its mammalian homologues have the longest known C-termini, while the *Shab* protein has the longest N-terminus among all known Kv channels. Alternative RNA splicing can contribute substantially to diversity in the lengths of these regions, the C-terminus of Kv3.3 (for example) varying more than twofold in length in alternate forms.

While this diversity of structure suggests a corresponding diversity of function, relatively little is known about the specific roles of the cytoplasmic "tails" of these proteins. In some channels (e.g., *Shaker* and Kv1.4), an N-terminal peptide has been shown to participate in rapid inactivation, although

FIGURE 12(3).

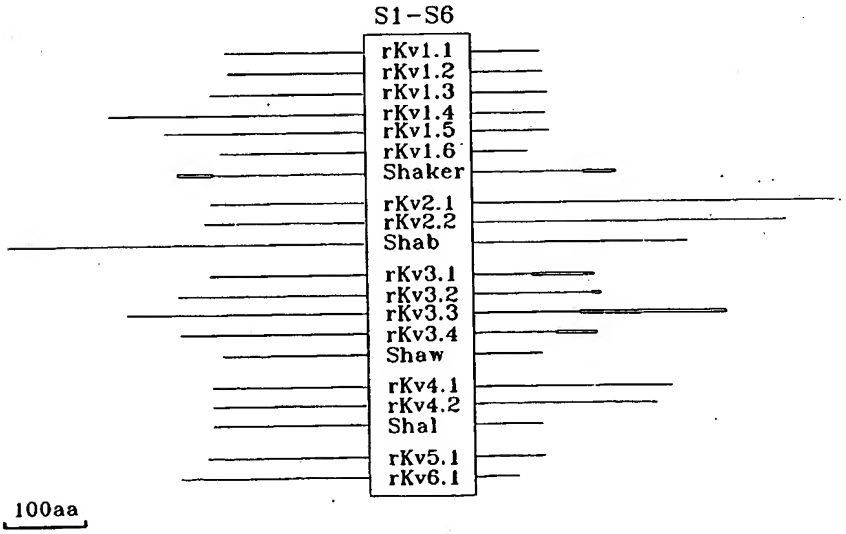

FIGURE 13. Schematic diagram showing the relative lengths of the N-terminal and C-terminal cytoplasmic "tails" of Kv proteins. The boxed region represents the hydrophobic core of the proteins from S1 through S6, and size differences within this region have been ignored. Those segments known to vary in length through alternative mRNA splicing are indicated by open boxes. The scale bar indicates a length of 100 residues.

there is relatively little sequence conservation of such peptides. The strongly conserved region in the N-terminal cytoplasmic region of these proteins (the region including the YFFDR motif) could be important in establishing the specificity of subunit interactions in its native tetrameric form (Li et al., 1992; Shen et al., 1993). Another (short) conserved motif is represented by the two adjacent cysteines present in the N-terminal region of all Kv genes other than the *Shaker* subfamily (IYLES*CC*QARY in rKv2.1, at position 382 in Figure 12), in a region with no homologue in the *Shaker* subfamily; a conserved cysteine is also located about 25 residues upstream in this same group of proteins (HMMEEM*C*ALS in rKv2.1, at position 361 in Figure 12), although no function has been ascribed to any of these cysteines. Two additional conserved cysteines, one in S2 and the other in S6 (see Figure 12), when mutated in Kv2.1, did not affect channel function (Zuhlke et al., 1993).

2.1.3.2 The P-Region

Portions of the P-region (spanning some 22 residues) are among the most highly conserved sequences within this family of proteins. In fact, as illustrated in the alignment in Figure 14, this region can be identified in *all* known K^+-selective channels, including all Kv channels (which we have discussed here), calcium-activated K^+ channels of fly and mouse (Atkinson et al., 1991; Butler et al., 1993), inward rectifier K^+ channels in plants and mammals (Anderson et al., 1992; Sentenac et al., 1992; Ho et al., 1993; Kubo et al., 1993a,b) including the G-protein-coupled rat GIRK channel (Kubo et al., 1993b), the fly *eag* K^+ channel (Warmke et al., 1991, 1993), and a putative K^+ channel isolated from *E. coli* (Milkman, 1994; also see Table 1).

```
Shaker          NSFFKSIPDAFWWAVVTMTTVGYGDMTPVGFWGKIV
rKv1.1(RCK1)    E-H-S----------=---------Y--TIG----
Shab            D=K-V---=-----G=----------=C-TTAL--==
rKv2.1(DRK1)    D=K-----AS----T=----------=Y-KTLL----
Shaw            HND-N---LGL---=----------A-KT=I-MF-
rKv3.1(KV4)     H=H--N--IG----------=-----Y-QT=S-M=-
Shal            A-K-T---A---=T=-----=-----V-KTIA---F
mKv4.1(mShal)   K=N-T---A---=T=-----=-----V-STIA---F
rKv5.1(IK8)     E=L-----QS----==---------=Y-KTTL--=N
rKv6.1(K13)     SPE-T---AC=----=----------V-RSTP-Q=-
                  F sIP  fWwa vtMTTvGYGDm P   G      Kv consensus
ECOKCH          -PRIE-=MT--==S=E--=-------=V--SESA==F
slo             -AHRL-YWTCV==L=---=-------=YCETVL-=TF
mSlo            -NQAL=YW=CV=LL=---=-------=YAKTTL-==F
KAT1            A-L=NRYVT-L=-S=T-=--T----FHAENPREM=F
AKT1            E-L=MRYVTSM=-S=T-=--------=H--NTKEM-F
ROMK1           VENING=TS--L=S=E-QV-=---FRFVTEQCATA=
IRK1            V-EVN-FTA--L=S=E-Q--=---FRCVTDECPIA-
GIRK1           VANVYNF-S--L=F=E-EA-=---YRYITDKCPEG=
eag             P-RKSMYVT-L==T=TC--=--=-N=AAETDNE-=F
                          tmttvGyGd                  all K+ consensus
```

FIGURE 14. Amino acid sequence alignment of the putative pore region of K^+ channel proteins (see text). The symbol "–" indicates identity with the topmost sequence, while "=" indicates a conservative substitution. The consensus sequences for either the Kv family alone (below rKv1.6), or *all* K^+ channels (bottom), represent positions that either are completely conserved (upper case) or at which a single amino acid predominates (lower case).

The region aligned in Figure 14 contains 13 residues that are identical in all Kv channels, and an additional 5 that show only conservative changes within this group. Among the larger group of all K⁺-selective channels, only two residues are perfectly conserved *(G[Y/F]G),* and an additional three positions show exclusively conservative changes. In fact, the *eag* channel is the only known K⁺-selective channel that lacks the tyrosine in the otherwise universally conserved *GYG* motif, replacing it with the very similar phenylalanine; the recent demonstration that this channel is also permeable to Ca^{2+} (Bruggemann et al., 1993) could be relevant in this regard, although the Ca^{2+} permeability of other K⁺ channels has not been systematically examined. It should be noted that Isk/Mink channel lacks *any* sequence motifs resembling the K⁺ channel pore; however, a recent report by Attali et al. (1993) suggests that Isk may not be a channel at all, but rather an activator of endogenous K⁺ or Cl^- channels present in *Xenopus* oocytes.

The presence of this pore motif in both eubacteria and vertebrates suggests that its evolution predated the early branching of the "tree of life" more than three billion years ago. The fact that other cation channels (e.g., Na^+ and Ca^{2+}) do not share this motif suggests that the P-region plays a key role in defining the K⁺ selectivity of these channels, and that acquisition of K⁺ selectivity was an ancient evolutionary development.

The loop linking S5 to the P-region is thought to form part of the outer vestibule of the ion conduction pathway (Durell and Guy, 1992), since residues in this region have been reported to be involved in toxin binding (MacKinnon et al., 1989, 1990; Hurst et al., 1991; Goldstein et al., 1992). This loop is more highly conserved in its length than in its sequence, being 13–15 amino acids long in all but the mammalian *Shaw* homologues which have 22 residues (Figure 12).

2.1.3.3 The S4 Segment and Leucine Zipper

The S4 segment of K⁺ channels is thought to form a major element of the voltage sensor, and mutagenesis of its positive charges (and some hydrophobic residues) has been shown to alter the gating properties of *Shaker* (Papazian et al., 1991; Liman et al., 1991; Koren et al., 1990; Logothetis et al., 1992, 1993; Lopez et al., 1991; also see review by Jan and Jan, 1992). The S4 motif with its characteristic pattern of positive charges every third residue is also present in Ca^{2+} and Na^+ channels (Catterall, 1988), supporting its importance for voltage sensing. The presence of a similar motif in cGMP-gated channels seems to be at variance with their ligand-gated properties, but may account for their moderate voltage dependence (Kaupp et al., 1989; Ludwig et al., 1990).

The S4 segment, as indicated in Figure 12, is generally assumed to be about 20 amino acids in length, consistent with the presumed length of α-helix required to span the cell membrane. Given this disposition, the S4 segments of the *Shaker*-subfamily channels contain seven conserved positive charges (five arginines, two lysines) in the repeated motif, while the *Shaw*-subfamily proteins have six, and *Shab*-subfamily channels as well as Kv5.1 (IK8) and Kv6.1 (K13) each has five. Alignment of the *Shal*-subfamily

channels with the other Kv channels reveals a three amino acid deletion in the S4 segment, which eliminates the second arginine in the repeated motif, and in place of the last lysine they contain a glutamine, leaving them with only five positive charges. However, the *Shal* proteins contain an additional downstream arginine in phase with the repeated motif (at a position at which *Shaker* and *Shab* channels have a glutamine), and inclusion of this residue in the S4 segment would bring the total number of positive charges to six. If the S4 segment is of the same length in these different channels, then this sixth positively charged residue would be included in the voltage sensor of the mammalian *Shal*-subfamily proteins, as well as in the fly *Shaw* protein. Nor has the possibility been excluded that the arginine at this position in the mammalian *Shaw* proteins also participates in voltage sensing.

Voltage sensing in these channels certainly depends on structural features outside the S4 region itself. While specific residues outside S4 influencing this process have not yet been identified, charged amino acids at highly conserved positions may be attractive candidates. It is tempting, for example, to suggest that the conserved arginine present in the S2 segment of all Kv protein may influence the voltage dependence of activation of these channels. Several negatively charged residues are also conserved in all Kv channels (the FFDR motif in the N-terminal region already noted, a glutamic acid in S2, an aspartic acid in S3, and a glutamic acid at each end of S5); these and other charged residues could influence voltage sensing in the various Kv isoforms.

A leucine heptad motif is repeated five times at the end of the S4 segment and S4/S5 loop of all the *Shaker*- and *Shab*-subfamily channels (Figure 12), forming a "leucine zipper" motif thought to be important in many protein–protein interactions (McCormack et al., 1989; Alber, 1992). Leucines 2 and 5 (L2 and L5) are present in all Kv channels. L1 is also found in all the channels, with the exception of the *Shal*-subfamily channels, which have a phenylalanine at that position. Similarly, phenylalanine replaces L4 in the *Shaw* channels and Kv6.1, while phenylalanine and alanine are present in *Shaw* and Kv6.1 in the place of L3. Substitutions of these leucines in the *Shaker* channel has been shown to alter the voltage-dependent steps during channel gating (McCormack et al., 1991; Schopa et al., 1992). While the significance of this leucine zipper-like structure is still not understood, it clearly can influence channel gating, perhaps through modification of interdomain interactions.

2.1.3.4 Kv-Channel Phylogenetic Tree

Figure 15 shows a proposed phylogenetic tree for the extended Kv family. This topology was determined by first examining trees including only members of the Kv1, Kv2, Kv3, and Kv4 subfamilies, and constraining the relationships within the *Shaker* and *Shaw* groups to be those shown in the trees in Figures 4 and 10; the most parsimonious tree found in this way makes neighbors of the *Shaker* and *Shal* groups on the one hand, and the *Shaw* and *Shab* groups on the other, as in our earlier analyses (Strong et al., 1993).

FIGURE
based
progran

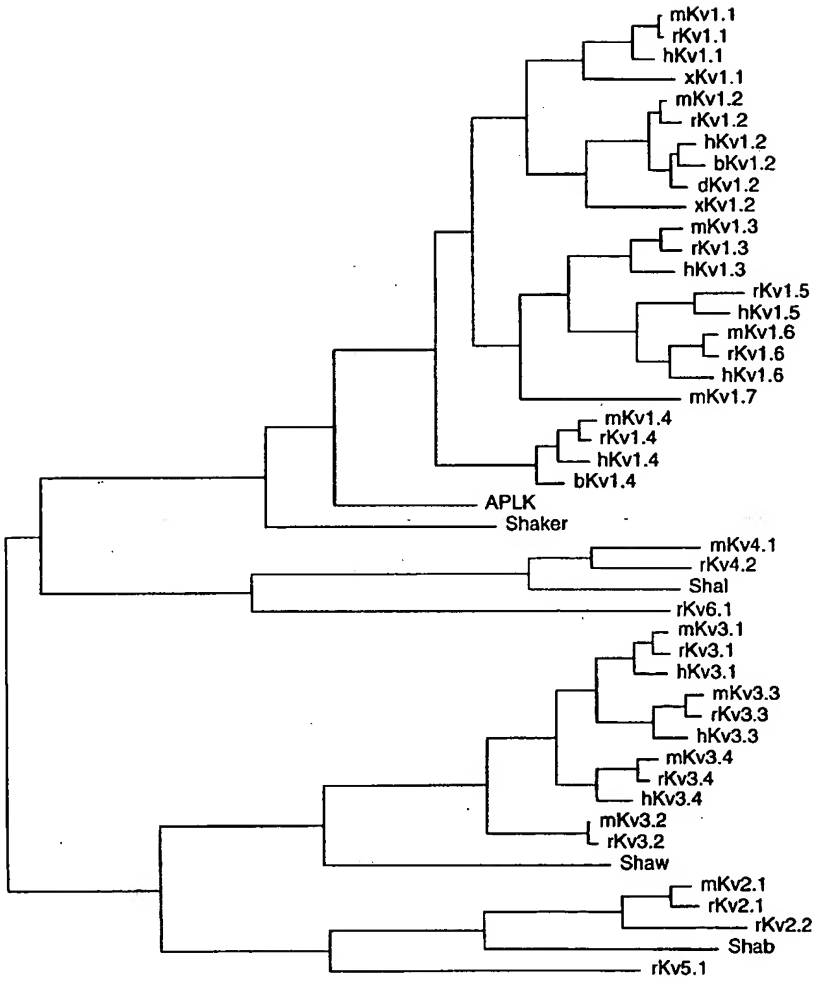

FIGURE 15. Proposed phylogenetic tree of voltage-gated K⁺ channel genes, based on parsimony analysis of nucleotide sequence alignments using the program PAUP (Swofford, 1993; see text).

Adding rKv5.1 (IK8) and rKv6.1 (K13) to such a tree places the former on the branch leading to *Shab*, and the latter on that leading to *Shal*, as shown in the figure. However, neither the deep branching between the Kv1 and Kv4 subfamilies in this tree (*Shak/Shal* versus Shab/Shaw), nor the placement on this tree of Kv5.1 and Kv1.6 is compellingly supported by bootstrapping. The deep divisions within this group of genes, together with their as yet unresolved rooting (Strong et al., 1993), may therefore have to await the availability of additional sequence data, or more powerful analytic methods, for their satisfactory resolution.

Table 2a. Tissue Distribution of Voltage-Gated Potassium Channel Genes

	Expression in tissue[a]															
Gene	**Brain**	**Atr**	**Vent**	**Kid**	**Retina**	**Lung**	**Liver**	**Skm**	**Islet**	**Thy**	**Spl**	**Lym**	**C_2C_{12}**	**GH3**	**Aorta**	**Comments**
Kv1.1	+[1,2]	+[3]	−[3]		+[4]		−[3]	+[5]	+[6]				−[7]		−[3]	Olfactory bulb +[1]
Kv1.2	+[1,2]	+[3,8]	+[3,8]				−[3]	−[3]	+[6]				−[7]		−[3]	Atrium > vent,[3] NG108-15 +[9]
Kv1.3	+[1]	−[10,11]	−[10,11]	−[10,11]		+[11]	−[10,11]	−[11]	+[6]	+[12,13]	+[10,11]	+[10–15]	−[7]			Fibroblasts +,[10] pre-B +[16]
Kv1.4	+[1,17]	+[18]	+[18]	−[19]			−[3]	+[7]	+[6]				+[7]	+[20]	−[19]	Atrium = vent,[18] 2.3 kb mRNA in skm;[7] adrenal medulla +, stomach −[9]
Kv1.5	+[22]	+[3,11]	+[3,11]	+[11]		+[11]	−[11]	+[7]	+[23]				−[7]	+[24]		Ant. pituitary +,[24] hypothalamus +[24] 3 kb mRNA skm[7]
Kv1.6	+[25]	−[22]	−[22]	−[22]		−[22]	−[22]	−[22]	+[6]							
Kv2.1	+[25,26]	+[25,26]	+[25,26]	+[25,26]	+[25,26]	−[22]	−[22]	+[25,26]					+[7]			Retina[26]>kid[26]>vent[26] >skm[25]>atrium[25] >olfactory epithelium[25] >tongue[25]
Kv2.2	+[26]	−[26]	+[26]		+[26]		−[26]	−[26]								Tongue[26]>olfactory epithelium[26] >ventricle[26]>retina[26]
Kv3.1	+[27]	−[28]	−[28]	−[28]		−[28]	−[28]	+[7]			+[29]	+[29]	+[7]			Human Louckes B-cells +[29] NG108-15 +,[9] AtT20 +[30]
Kv3.2	+[31]	−[22]	−[22]	−[22]				−[22]								
Kv3.3	+[32,33]						+[33]									
Kv3.4	+[34]							+[34]								
Kv4.1	+[7,35]							−[7]					+[7]			
Kv4.2	+[17]	+[3]	+[3]				−[3]	−[3]							+[3]	Atrium = vent[3]
Kv5.1	+[22]	−[22]	−[22]	−[22]		+[22]	+[22]	−[22]								
Kv6.1	+[22]	−[22]	−[22]	−[22]	−[22]		−[22]	−[22]								

[a] Atr, heart atrium; Vent, heart ventricle; Kid, kidney; Skm, skeletal muscle; Islet, pancreatic islet; Thy, thymus; Spl, spleen; Lym, lymphocytes; C_2C_{12}, C_2C_{12} cells in culture; GH3, GH3 cells in culture.

Table 2b. Dist

Gene	
Kv1.1	Pons/n.
Kv1.2	[CA3: Pons/n CA1], olfact
Kv1.3	Inferio striatu
Kv1.4	Olfactc >cerel
Kv1.5	Cerebe
Kv1.6	Medull
Kv2.1	Cerebr
Kv2.2	Olfactc region
Kv3.1	Cerebe cortic while embry
Kv3.2	Thalan
Kv3.3	Cerebe
Kv3.4	Brain[34]
Kv4.1	Brain[1P]
Kv4.2	Cerebe thalan
Kv5.1	Brain[22]
Kv6.1	Brain[22]

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Table 2b. Distribution of Voltage-Gated Potassium Channel Genes in Mammalian Brain

Gene	Comments
Kv1.1	Pons/medulla[1]>mid brain,[1] superior and inferior colliculus,[1] cerebellum[1] >hippocampus [CA3>dentate gyrus>CA1],[1,2] thalamus,[2] cerebral cortex[1,2] >corpus striatum,[1] olfactory bulb[1]
Kv1.2	Pons/medulla[1] > cerebellum,[1,2] inferior colliculus[1] >hippocampus [CA3>dentate gyrus = CA1],[1,2] thalamus,[2] cerebral cortex,[1,2] superior colliculus[1] >mid brain,[1] corpus striatum,[1] olfactory bulb[1]
Kv1.3	Inferior colliculus[1] >olfactory bulb,[1] pons/medulla[1] >mid brain,[1] superior colliculus,[1] corpus striatum,[1] hippocampus,[1] cerebral cortex[1]
Kv1.4	Olfactory bulb,[1] corpus striatum,[1] > hippocampus,[1,17] superior and inferior colliculus[1] >cerebral cortex,[1] mid brain,[1] basal ganglia[17] > pons/medulla[1]
Kv1.5	Cerebellum [purkinje and granular cells],[22] hypothalamus[24]
Kv1.6	Medulla/pons[25] > inferior colliculus[25] > corpus striatum[25]
Kv2.1	Cerebral cortex[26] > hippocampus[22] > cerebellum[22] > olfactory bulb[22]
Kv2.2	Olfactory bulb [granule cell layer>olfactory tubercle][26] >cortex[26] >hippocampus [CA1–CA4 region, dentate gyrus][26] >cerebellum[granule cell layer][26]
Kv3.1	Cerebellum[27] >globus pallidus,[27] subthalamus,[27] substantia nigra[27] > reticular thalamic nuclei,[27] cortical and hippocampal interneurons[27] >inferior colliculi,[27] cochlear and vestibular nuclei;[27] while Kv3.1a is expressed mainly in the adult brain Kv3.1b is the major transcript in embryonic and perinatal neurons; their distribution within the brain is identical[27]
Kv3.2	Thalamus[31] >neocortex [layers 4–6],[31] piriform nucleus,[31] red nucleus,[31] hippocampus [CA3][31]
Kv3.3	Cerebellum [purkinje cells][32,33]
Kv3.4	Brain[34]
Kv4.1	Brain[18,35]
Kv4.2	Cerebellum [granular cells][17] >hippocampus[granule cells, CA3/CA1 pyramidal cells],[17] thalamus,[17] medial habenular nucleus[17] >cerebral cortex[17]
Kv5.1	Brain[22]
Kv6.1	Brain[22]

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2.1.4 Tissue Distribution of Kv Channels

2.1.4.1 Tissue-Specific Expression of Kv Genes

Although all known Kv genes are expressed in the brain, their distribution in other tissues is restricted (Table 2a). Even within the brain the localization of each of these genes is highly restricted and variable (Table 2b; e.g., Rudy et al., 1992a). The molecular mechanisms underlying these tissue-specific expression patterns are not understood. For the sodium channel genes, particular silencer and enhancer elements govern the tissue-specific expression of these genes in brain and/or skeletal muscle (Maue et al., 1990; Mori et al., 1990; Z.-H. Sheng et al., 1992). Future studies may determine whether similar mechanisms are responsible for the restricted tissue-distribution patterns of the Kv genes.

Kv channels can also show very specific patterns of subcellular localization. Although Kv1.4 and Kv4.2 mRNAs are both expressed in the same neuronal cells, Kv1.4 protein is found in axons and possibly nerve terminals, while Kv4.2 is mainly concentrated in dendrites and soma (M. Sheng et al., 1992). Kv2.1 also segregates to the cell body and dendrites of rat brain neurons (Trimmer, 1991). The processes that determine subcellular distribution patterns of channel proteins remain to be defined.

2.1.4.2 Developmental Regulation and Induction

In addition to their unique tissue distribution, Kv channel expression is tightly regulated during differentiation and in mature cells, several examples of which are listed below. Kv3.1 mRNA expression is first evident in the cerebellum of rats, 11 days postnatal, and continues to increase throughout adulthood (Drewe et al., 1992). In the mouse pituitary cell line AtT20, transfection of the human H-*ras* oncogene results in a six-fold enhancement of a TEA-sensitive voltage-gated K^+ current (presumably Kv3.1), and a parallel induction of Kv3.1 mRNA expression (Hemmick et al., 1992; Perney et al., 1992). Kv3.1 mRNA expression can also be triggered in these cells by a short depolarizing pulse (via an influx of extracellular calcium), as well as by basic fibroblast growth factor (Perney and Kaczmarek, 1993). Kv1.5 transcripts are down-regulated during the development of the rat ventricle, completely disappearing by 6 months, whereas atrial expression of this gene remains unaltered (Matsubara et al., 1991); dexamethasone induces Kv1.5 mRNA expression in clonal pituitary cell lines (Levitan et al., 1991; Takimoto et al., 1993), whereas cAMP reduces expression of these mRNAs in the same cells (Mori et al., 1993). The dexamethasone-induced increase in Kv1.5 mRNA is a consequence of enhanced transcription rather than a reduction in mRNA turnover (Takimoto et al., 1993). Pentylenetetrazole-induced seizures in rats are accompanied by a reduction of Kv1.2, Kv4.2, and Kv3.1 mRNA (Tsaur et al., 1992; Perney and Kaczmarek, 1993). Of the three Kv2.1 transcripts (4.3 kb, 9 kb and 10 kb), only the 10-kb mRNA is induced by nerve growth factor in the rat pheochromocytoma, PC12 (Rudy et al., 1992b). Lastly, rKv1.5 mRNA expression increases in cAMP-dependent manner in neonatal atrial cells, and a canonical cAMP-response element (TGACGTCA) located 435 bp downstream to the transcription start site may be responsible for this modulation (Matsubara et al., 1992; Mori et al., 1993).

Many of the Kv genes have multiple transcripts that may be selectively expressed in a particular tissue, providing an additional level of complexity to the system. For example, the Kv1.4 gene generates three mRNAs (4.5, 3.5, and 2.4 kb), and only the 2.4-kb transcript appears to be expressed in skeletal muscle and in the mouse myoblast cell line, C_2C_{12} (Lesage et al., 1992). The molecular mechanisms underlying the tissue-specific expression of different mRNAs have not yet been defined.

We have already discussed posttranslational modifications (phosphorylation and glycosylation) that may modulate Kv function, and such processes may be important in the induction and developmental regulation of Kv channel expression. Diverse cell lines can begin expressing functional voltage-gated K^+ currents within minutes after being exposed to low-dose radiation, heat-shock, or free radicals (Kuo et al., 1992). This process is not blocked by DNA or protein synthesis inhibitors, suggesting that nonfunctioning pools of Kv protein exist in these cells, and become functional in response to environmental stimuli. The recent generation of specific antibodies against several Kv channels may facilitate studies to delineate the nature of those posttranslational events required for expression and regulation of Kv channels (Barbas et al., 1989; Trimmer, 1991; Schwarz et al., 1990; M. Sheng et al., 1992; Spencer et al., 1993; Perney et al., 1993).

2.1.5 Structural and Electrophysiological Analyses of Kv Channels

2.1.5.1 Tetrameric Structure of Functional Kv Channels

Homomultimers of the single domain structures described above form functional channels, and some of these (Kv1.3, Kv1.4, Kv1.5, Kv3.1) resemble specific native channels found in mammalian cells (discussed above). Coexpression of two kinetically or pharmacologically distinct K^+ channels results in ionic currents with hybrid behavior (Christie et al., 1990; Isacoff et al., 1990; Ruppersberg et al., 1990; K. McCormack et al., 1990) indicating that heteromultimeric channels may contribute to functional diversity. In fact, the existence of functional heteromultimers of *Shaker*-related peptides has recently been demonstrated in brain and myelinated nerves of rats and mice (Sheng et al., 1993; Wang et al., 1993). However, Kv proteins appear to be capable of associating only with other Kv members of the same subfamily (Covarrubias et al., 1991).

The α subunits of voltage-gated sodium and calcium channel contain four homologous domains that are thought to assemble around a central pore to form a functional channel. Each of these domains is clearly homologous to the product of a Kv gene, and the functional K^+ channel has therefore been proposed to be a tetramer. This proposed model has been experimentally supported by analysis of heteropolymers formed from K-channel domains with differing drug sensitivities (MacKinnon, 1991b; Liman et al., 1992). Constructs containing four K-channel domains linked in tandem can produce functional channels (Liman et al., 1992), also supporting this model. A 115 amino acid region in the amino-terminus of the *Shaker* channel (residues 82–196) has been reported to contain determinants responsible for tetramer formation, and deletion of this region from the *Shaker* channel abolishes expression of the K^+ current (Li et al., 1992; Shen et al., 1993). However, deletion of the corresponding segment from mKv1.3 (Aiyar et al., 1993b) or rKv2.1 (VanDongen et al., 1990) does not affect channel function, implying

Table 3. Electrophysiological and Pharmacological Properties of Cloned Kv Channels

			Pharmacology, 50% inhibitory concentrations[a]						
Channel	Activation V_n (mV)	Single channel currents (pS)	TEA (mM)	CTX (nM)	DTX (nM)	MCDP (nM)	4-AP (mM)	Quinine (μM)
mKv1.1	−27,[1–34][2]	10[2]	0.4,[1,12]0.3[2]	>1500[2]	21[2]	490[2]	1.1,[1]0.29[2]	41[2]
rKv1.1	−30[3,4]	9,[3,5]14.2[6]	0.6,[3,5]0.8[4]	22[3]	12[3,5]	45[3,5]	1.0,[3,5]0.16[4]	>100[4]
hKv1.1	−30[7]	?	20[7]	>100[7]	?	?	1.1[7]	?
rKv1.1	+3,[2–34,]3+4.8[8]	18[2,9]	>560,[2]129,[3]10[9]	17,[76,]1.7[8]	24,[4,]2.8[8]	440,[7]180[3,8]	0.6,[3]0.8,[3]0.2[9]	
800,[9]600[10]			>50[7]	10[7]	?	?	0.8[7]	?
hKv1.2	−5[7]	?	11[2,11]	2.6,[7]0.5–2.0[11]	250[2]	>2000[2]	0.20,[7]0.4[11]	40[11]
mKv1.3	−23,[2–35][11]	14,[7]13[11]	50,[2]11,[12]>40[13]	1[3]	>500[3,12]	>1000[3]	1.5[3]	80[12]
rKv1.3	−25,[3–14][12]	9.6[3]	14,[14]30[15]	0.8[15]	?	?	0.19,[14]0.3[15]	55[14]
hKv1.3	−13,[14–20][15]	13[16]	>1600[38]	>1000[38]	?	?	?	22[38]
mKv1.4	−27[38]	?	>100[3]	>40[3]	>200[3]	>2000[3]	13,[3]1.2[17]	?
rKv1.4	−22[3]	4.7[3]	>50[7]	?	?	?	0.8,[7]0.7[18]	?
hKv1.4	−5,[7–34][18]	?	>40[23]	>200[23]	>200[23]	>600[23]	0.4[13]	?
rKv1.5	−13,[9–3][13]	7.9[19]	330,[2]>40[20]	>1000[2]	>1000[2]	>10000[2]	0.27,[2]<0.1[20]	?
hKv1.5	−25[2]	8[2]	4,[17,]11.7[22]	1,[21]>3000[22]	20,[21]25[22]	10,[21]200[22]	1.5,[21]0.3[22]	?
rKv1.6	−13,[13–17][21]	8.7,[21]8.2[22]	7[21]	1[21]	20[21]	10[21]	1.5[21]	?
hKv1.6	−21[21]	9.1[21]	5[23]	?	?	?	>100[23]	?
mKv2.1	−14[23]	?	10,[24]5.6[25]	>1000[24]	?	?	0.5[24]	?
rKv2.1	−9.2[24]	8.1[24]	7.9[26]	?	?	?	?	?
rKv2.2	?	?	0.1[27]	?	?	?	0.6[27]	1000[27]
mKv3.1a	+16[27]	26[27]	0.15[2,28]	>1000[2]	>1000[2]	>2000[2]	0.021,[7]0.18[28]	4–20[2,28]
mKv3.1b	+21,[2–1][28]	27[2,28]	0.1,[29]0.15[30]	>1000[30]	>100[29]	>1000[30]	0.25,[29]0.15[30]	1000[29]
rKv3.1b	+19,[29+15][30]	22,[24]11[29]	0.1,[29]<1[31]	?	>100[29]	>200[29]	0.9,[29]<1[31]	130[31]
rKv3.2	+5.6,[29+13][31]	11[29]	0.14[32]	?	?	?	1.2[32]	?
rKv3.3	+7[32]	?	0.3[29,33]	?	>100[29,33]	>200[29]	0.5[29,33]	500[29]
rKv3.4	−3.4,[29+14][33]	14[33]	0.088[34]	?	?	?	0.6[34]	?
hKv3.4	+19[34]	?	>10[35]	?	?	?	9[35]	?
mKv4.1	0[35]	?						

Table 3. (Continued)

TEA, tetraethylammonium; CTX, charybdotoxin; DTX, dendrotoxin; MCDP, mast cell degranulating peptide; 4-AP, 4-aminopyridine. Reported values are rounded to two significant figures, and comparable values differing by less than 10% are shown as identical.

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that other residues, presumably in the hydrophobic core, are responsible for assembly of mammalian K^+ channel multimers. Recent electron microscopic analysis of purified K^+ channel protein has begun to provide provide direct confirmation of its tetrameric structure (Lie et al., 1994).

2.1.5.2 Biophysical Properties of Cloned Kv Channels

Table 3 summarizes some of the electrophysiological and pharmacological properties of the channels encoded by each of the cloned Kv genes. Of the 16 mammalian Kv channels that have been biophysically characterized, only five (Kv1.4, Kv3.3, Kv3.4, Kv4.1, and Kv4.2) display rapid (A-type) inactivation, and the remainder are delayed rectifiers (Table 3). Many of these measurements have been made on whole oocytes with the two-voltage clamp or the macropatch methods. With the availability of a variety of cell lines stably expressing transfected channel genes, which are listed in Table 4, it has now become possible to study the biophysical properties of the cloned channels with traditional patch clamp recording techniques. It is also possible to perform patch clamp measurements on membrane patches isolated from oocytes expressing channel genes.

One must exercise considerable caution in interpreting the results of such studies, however, as the results obtained with these different methods are not always in agreement, even for identical channels. For example, the single K^+ conductance for rKv1.1 was 9 pS when measured in oocytes with the macropatch method, whereas studies on a fibroblast cell line stably expressing this gene revealed a single K^+ conductance of 14.2 pS (see Table 3). Another difference between oocyte and patch clamp experiments is in the resulting measurements of sensitivity to TEA, quinine, and verapamil. For example, the TEA sensitivity of Kv1.3 is lower in intact oocytes (K_d = 50 m*M*) than in patches (K_d = 11 m*M*). In addition, the time course of inactivation measured in whole oocytes is much slower than in oocyte patches or in mammalian cells (Grissmer et al., 1992). The larger single K^+ conductance measured in mammalian cells may in part be due to the higher external K^+ concentration in mammalian Ringer (4.5 m*M*) versus *Xenopus* Ringer (2 m*M*). The differences in pharmacological sensitivity and time course of inactivation may result from involvement of cytoplasmic factors (Marom et al., 1993). One must also consider the overall level of channel expression when interpreting oocyte data, since qualitatively different K^+ currents may be generated by different levels of a single cRNA (Honore et al., 1992). Lastly, grossly conflicting results (such as the CTX sensitivity of rKv1.6 shown in Table 3) can result from contaminated toxin preparations (MacKinnon and Miller, 1989; Garcia-Calvo et al., 1992); this is discussed further below.

2.1.5.3 Relating Cloned Kv Channel Genes to Native K^+ Channels

One goal of molecular biological studies on K^+ channels is to identify those Kv genes that encode known native channels found in normal cells. A commonly used approach has been to compare the biophysical and pharmacological properties of each of the cloned channels with particular native channels. Unfortunately, the quantitative differences in the measurements made by the two-electrode recording technique in whole oocytes, compared with those made by the patch clamp on native mammalian cells, has complicated comparisons of this type. Patch clamp experiments on mammalian cell lines that stably express Kv genes (Table 4), or that have been injected with Kv-cRNA (Ikeda et al., 1992), may be helpful in this regard. Another factor that complicates such comparisons is that native channels may be composed of products of more than one Kv gene. The formation of functional heteromultimeric channels within the mammalian *Shaker*-related subfamily had been shown in *Xenopus* expression systems (Ruppersberg et al., 1990; Isacoff et al., 1990), and their existence has recently been demonstrated in rat brain (Sheng et al., 1993; Wang et al., 1993).

Table 4. Stable Expression of Transfected Kv Channels in Cultured Cells

Channel	Cell line
mKv1.1	CHO cells,[1] L929[2]
rKv1.1	Sol-8 myoblasts,[3] mouse L cells,[4] MEL (mouse erythroleukemia) cells[5]
rKv1.2	B82 mouse fibroblasts[6]
mKv1.3	A4[2]
hKv1.3	IM-9 B cells[7]
bKv1.4	Neuro-2A[8]
hKv1.4	MEL cells[5]
hKv1.5	CHO cells,[9] MEL cells[5]
mKv3.1	Human embryonic kidney cells (293),[10] L929 cells[2]

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2.1.5.3.1 Mammalian Kv Channels

Only five cloned Kv genes have thus far been convincingly related to native mammalian channels. Kv1.3 and Kv3.1 encode the well-characterized types *n* and *l* K^+ channels in lymphocytes and the Kv1.5 gene encodes a rapidly activating delayed rectifier in human heart, both discussed further below. The

cloned Kv1.4 channel has properties indistinguishable from a K^+ channel in GH3 pituitary cells, and Kv1.4 cDNA has been isolated from these cells, suggesting that the native channel is a homomultimer formed from Kv1.4 subunits (Meyerhoff et al., 1992). Similar considerations suggest that Kv1.1 encodes a delayed rectifier in glial cells (Wang et al., 1992).

Lymphocyte Channels. Since several reviews (e.g., Cahalan et al., 1991; Chandy et al., 1993) have described the properties and roles of three distinct voltage-gated K^+ channels, types *n, n′,* and *l,* expressed in T and B lymphocytes, this subject is only briefly discussed here. The Kv1.3 gene encodes the *n*-type channel while the *l* channel is a product of the Kv3.1 gene (see Chandy et al., 1993). Both native channels are probably homomultimers, since their properties are nearly identical to that of their cloned counterparts (Grissmer et al., 1990, 1992; Douglass et al., 1990; Attali et al., 1992b). The gene encoding the type *n′* channel has not been identified. Blockade of Kv1.3 channels in T cells by chemically disparate pharmacological agents (see Table 3), including highly selective toxins (margatoxin, noxiustoxin, and CTX), results in membrane depolarization, diminution of the calcium signal, and suppression of the lymphocyte activation cascade (reviewed in Chandy et al., 1993). Drugs that selectively block Kv1.3 could therefore be developed into immunosuppressants for use in prevention of graft rejection and inflammation. The restricted tissue distribution of Kv1.3 (Table 2) adds to its attractiveness as a target for immunosuppression. These features have prompted several pharmaceutical groups to actively search for novel compounds that block Kv1.3 channels with a high degree of potency and selectivity. Interestingly, human platelets (Mahaut-Smith et al., 1990) and the melanoma cell line IRG1 (Nilius et al., 1990) express a CTX-sensitive voltage-gated K^+ channel, which is remarkably similar to the Kv1.3 channel.

Cardiac Channels. Several delayed rectifier Kv channels (Kv1.1, Kv1.2, Kv1.5, Kv1.6, Kv2.1, Kv2.2,) are present in the heart (see Table 2). As mentioned above, a rapidly activating delayed rectifier in the heart is most likely formed from four Kv1.5 subunits (Fedida et al., 1993). The remaining genes probably encode subunits of other types of cardiac I_k delayed rectifier channels that are responsible for repolarization during the cardiac action potential. Direct comparisons of the properties of these cloned channels and cardiac delayed rectifiers do not reveal any close similarities, suggesting that the native channels may be heteromultimeric.

A rapidly inactivating K^+ current, I_{to}, modulates the early plateau phase of the cardiac action potential. At least two A-type (rapidly inactivating) Kv channels, Kv1.4 and Kv4.2, are expressed in the heart (Table 2), while the expression in heart of the other three A-type channels, Kv3.3, Kv3.4, and Kv4.1, has not been examined. Like the I_{to} channel, Kv1.4 has small conductance (4.7 pS), is blocked by 4-AP, and is resistant to CTX, DTX, and external TEA (Po et al., 1992); in addition, the level of Kv1.4 transcript is elevated in ventricular hypertrophy (Matsubara et al., 1993), a condition associated with an increased I_{to} current. However, rat and human Kv1.4 recover from inactivation about 10-fold more slowly than the native I_{to} channel, suggesting that the native I_{to} channel may be heteromultimeric (Po et al., 1993). In fact, heteromultimeric channels formed by coinjection into oocytes of Kv1.4:Kv1.2 or Kv1.4:Kv1.5 cRNA show the rapid recovery from inactivation characteristic of the I_{to} channel (Po et al., 1993).

Drugs that modulate cardiac Kv channels have been successfully utilized in the treatment of ventricular arrhythmias. These class III antiarrhythmic agents (e.g., UK-68798 or Dofetilide, E-4031, Almokalant, and Sematilide) prolong myocardial refractoriness, and suppress reentrant atrial and ventricular arrhythmias in dog and pig models (see reviews by Lynch et al., 1992; Colatsky et al., 1993). The recent increase in our understanding of the molecular structure of cardiac Kv channels may lead to the identification of more selective and/or potent class III antiarrhythmics.

2.1.5.3.2 Drosophila Kv Channels

Shaker channels are expressed in diverse excitable cells in the fly, including muscle and photoreceptors (Solc et al., 1987; Hardie et al., 1991). The products of the different *Shaker* transcripts inactivate with different time courses, ShB1, for example, inactivating more rapidly than ShA1 (Timpe et al., 1988; Stocker et al., 1990; Iverson et al., 1988; Wittka et al., 1991). The biophysical properties of the ShB1 channel, expressed in oocytes, are similar to the channels found in pupal muscle (Timpe et al., 1988). However, Pongs (1992), in a recent review, points out the existence of subtle differences in properties (mean open-time duration, kinetics of inactivation, toxin sensitivity) between cloned *Shaker* channels and their native counterparts; he suggests that the unique properties of native channels may arise as a consequence of interactions between *Shaker* subunits and other proteins [e.g., unidentified β subunits or *ether-a-go-go (eag)* proteins].

2.1.5.3.3 *Aplysia* Kv Channels

The *Shaker*-related *Aplysia* K^+ gene AK01a (Pfaffinger et al., 1991) encodes a protein of 515 amino acids containing the conserved glycosylation (in the S1-S2 loop) and PKC (in the S4–S5 loop) consensus sequences (Figure 2). Its ~15-kb mRNAs are present in the central nervous system, heart, gill muscle, and buccal muscle. The channel demonstrates A-type inactivation, half-activates at +5 mV, is 4-AP sensitive, and resistant to 10 m*M* TEA [presumably because of the arginine in the TEA binding site (GYGDM*R*P); see discussion below], 200 n*M* CTX and 1 μ*M* MCDP. The AK01a channel most closely resembles the IA_{depol} current detected in R15 neuronal cells of the abdominal ganglion of *Aplysia* (Pfaffinger et al., 1991).

2.1.5.3.4 *Xenopus* Kv Channels

Two Kv genes have been isolated from *Xenopus.* The xKv1.2 gene (XSha2), a homologue of the mammalian Kv1.2 genes, is expressed in the spinal cord and brain, but not in skeletal muscle, heart, liver, or eye (Ribera, 1990). Like rKv1.2 channels, the xKv1.2 channel is a delayed rectifier that activates

positive to –20 mV, is ~70% blocked by 1 m*M* 4-AP and is resistant to TEA (Ribera, 1990). K^+ channels with similar properties are found in *Xenopus* spinal neurons (Ribera, 1990). Although the single K^+ conductance of xKv1.2 has not been determined (and therefore cannot be compared with channels in spinal neurons), the mammalian homologues of xKv1.2 (see Table 3) have single K^+ conductances of 15 to 30 pS, which resemble those of the delayed rectifiers in spinal neurons. The second channel, xKv1.1, shares 88% amino acid sequence identity with mKv1.1 and rKv1.1, and is expressed in primary spinal neurons (Rohon-Beard cells) and ganglia, trigeminal ganglia, and gill arches (Ribera and Nguyen, 1993). Like mammalian Kv1.1, the xKv1.1 gene (xSha1) encodes a delayed rectifier that activates positive to –40 mV and is ~60% blocked by 1.5 m*M* external TEA. The physiological and developmental roles of these channels remain to be elucidated.

2.1.5.4 Identifying the Residues That Underlie Channel Inactivation

At least three distinct parts of the protein (N-terminus, P-region, and S6 segment) contribute to channel inactivation. Rapid inactivation involving the N-terminus has been called "N-type" inactivation, alternative modes being referred to as "C-type" and "P-type" inactivation (Iverson and Rudy, 1990; DeBiasi et al., 1993a; Hoshi et al., 1991; Lopez-Barneo et al., 1993).

2.1.5.4.1 Rapid Inactivation Associated with the Amino-Terminus

Deletion of the NH_2-terminus from the *Shaker* A2 channel removes rapid inactivation (Hoshi et al., 1990), while addition of an N-terminal peptide (MAAVAGLYGLGED*R*QH*RKK*Q) to the inner surface of this channel restores inactivation (Zagotta et al., 1990). The four basic residues indicated within this segment are thought to form a tethered ball that interacts with a receptor elsewhere in the channel, possibly the S4–S5 linker (Isacoff et al., 1991), and substitution of glutamine for two or more of these basic residues slows down inactivation (Hoshi et al., 1991). The N-termini of the five mammalian A-type channels (Kv1.4, Kv3.3, Kv3.4, Kv4.1, Kv4.2) contain charged residues that may constitute a similar inactivation ball (see Figures 2, 8, and 11). Peptides corresponding to this region in Kv1.4 (RARERERLAHSR) and Kv3.4 (RGKKSGNKPPSKTCLK) have been shown to accelerate inactivation of these channels (Ruppersberg et al., 1991b); similar studies have not yet been carried out on Kv3.3, Kv4.1, and Kv4.2. Deletion of residues 3–25 from rKv1.4 abolishes N-type inactivation indicating that the ball might lie in this segment (Tseng and Tseng-Crank, 1992). A similar deletion (residues 2–32, including one arginine; Figure 11) from mKv4.1 slows down, but does not eliminate, rapid inactivation (Pak et al., 1991a). Deletion of three basic residues (PAPPRQE*RKR*TQ) further downstream in the N-terminus of rKv4.2 (Figure 11) also reduces N-type inactivation of this channel (Baldwin et al., 1991).

A modulatory site has been identified near the amino-terminus of mammalian A-type K^+ channels (Ruppersberg et al., 1991a). Rapid inactivation of the A-current channels, rKv1.4 and rKv3.4, completely disappears when inside-out patches are excised from oocytes expressing these genes, and can be restored by exposing the inner surface of these channels to the reducing agent glutathione (Ruppersberg et al., 1991a). This loss of inactivation is due to the oxidation of a cysteine located upstream to the charged ball in both rKv1.4 (SSG*C*NS) and Kv3.4 (SSV*C*VS) (Ruppersberg et al., 1991a). The *Shaker* channel lacks the regulatory cysteine and inactivation of this channel does not disappear in excised patches (Ruppersberg et al., 1991a). Since Kv3.3 also has a cysteine at this position (see Figure 8), inactivation of this channel might also be sensitive to oxidation.

2.1.5.4.2 The Receptor for the N-Terminal-Inactivating Ball

Mutations in the intracellular loop linking S4 and S5 of the *Shaker* channel (LQILGRTLKASMREL) alter inactivation kinetics suggesting that this region may be the receptor for the N-terminal ball (Isacoff et al., 1991; McCormack et al., 1991). For example, replacement of the conserved glutamate (MR*E*L) with either glutamine or aspartic acid dramatically slows down inactivation, whereas substitution of a conserved leucine (Q*L*GR) with either alanine or valine (amino acids with smaller hydrophobic side chains) accelerates inactivation (Isacoff et al., 1991). The leucine–alanine mutation also induces rapid inactivation of the normally noninactivating rKv2.1 (DRK1) channel (Isacoff et al., 1991).

The N-terminal ball is thought to cause inactivation by physically occluding the pore. Internal application of TEA dramatically slows N-type inactivation in the *Shaker* channel, probably by competing for a common binding site (Choi et al., 1991), and mutations of the conserved leucine and the glutamic acid in this region alter the TEA_i sensitivity of the *Shaker* channel (Slesinger et al., 1993). Collectively these data suggest that the receptor for the fast-inactivation ball must lie at, or close to, the cytoplasmic mouth of the ion conduction pathway. Once this site is occupied by the ball, the gating charges within the voltage sensor (presumably including S4 segment) may be immobilized and prevented from returning to its original position (Bezanilla et al., 1991).

Delayed rectifier and calcium-activated K^+ channels, despite the fact that they do not normally show rapid inactivation, appear to contain a receptor site for the *Shaker* inactivation ball, since the *Shaker* N-terminal peptide has been shown to induce rapid N-type inactivation of several of these channels (Zagotta et al., 1990; Dubinsky et al., 1992; Isacoff et al., 1991; Foster et al., 1992).

2.1.5.4.3 Inactivation Linked to the P-Region

In the rKv2.1 channel, removal of either the N- or C-terminus retards a slow form of inactivation called C-type inactivation, whereas rKv2.1 channels with both termini deleted behave like the wild-type channel (VanDongen et al., 1990). These studies suggest that residues within the hydrophobic core are responsible for slow inactivation. Recent studies point to residues within the P-region and S6 segment as being important for this type of inactivation

(DeBiasi et al., 1993a; Lopez-Barneo et al., 1993). Many of these experiments have been performed on *Shaker* channels with deleted termini (to remove N-type inactivation). In these truncated *Shaker* channels, replacement of threonine at the C-terminal end of the P-region (GYGDM*T*P) with glutamic acid or lysine (lysine occupies the position equivalent to threonine in Kv1.4), speeds up inactivation ~100-fold (Lopez-Barneo et al., 1993). Substitution of the threonine with tyrosine or valine (residues with large hydrophobic side chains) accelerates inactivation ~8-fold, while replacement with histidine (the homologous residue in Kv1.3) slows down inactivation ~5-fold (Lopez-Barneo et al., 1993). TEA, a K^+ channel blocker that interacts with residues at this site, also slows inactivation (Choi et al., 1991).

Similar experiments have been conducted on the rKv1.3 channel which has a histidine in place of the threonine present in the *Shaker* channel. Substitution of the histidine with a tyrosine reduces C-type inactivation roughly 5-fold, while replacement of the homologous tyrosine in rKv1.1 with histidine enhances inactivation ~2-fold (Busch et al., 1991).

Another site deeper within the P-region also influences inactivation. Replacing an isoleucine in the P-region of the rKv2.1 channel with leucine speeded up inactivation ~50-fold, and reduced mean open time at 0 mV, without affecting the single channel conductance (DeBiasi et al., 1993a). This type of inactivation has been termed P-type inactivation to distinguish it from C-type inactivation, which is slowed by external TEA (DeBiasi et al., 1993a).

2.1.5.4.4 Inactivation Linked to the S6 Segment

Two exons (exons 1 and 2), at the 3′-end of the *Shaker* coding sequence can be alternatively utilized to yield two channels, A1 and A2, which have distinct S6 segments and C-terminal regions. The two alternative S6 segments differ at only a single position, a valine in *Shaker* A1 (VGSLC*V*IAG) being replaced with an alanine in *Shaker* A2 (VGSLC*A*IAG). *Shaker* A1 currents show only rapid (N-type) inactivation, while *Shaker* A2 channels exhibit both N-type and C-type forms of inactivation (Wittka et al., 1991). Exchange of the residues at this position resulted in a transfer of inactivation properties (Wittka et al., 1991). All the mammalian *Shaker*-subfamily channels have alanine at this position (Figure 2) and could therefore be expected to exhibit slow inactivation as well.

2.1.5.4.5 β-Subunits and Inactivation

Two distinct β-subunits accelerate inactivation of voltage-gated sodium channels (Isom et al., 1992; also see review by Goldin in this volume). Similar Kv-associated small subunit proteins have been identified in rats (Rehm and Lazdunski, 1988), chickens (Schmidt and Betz, 1989), and cattle (Parcej et al., 1992). Low mol wt mRNA has been shown to encode a protein that can accelerate A-type inactivation of the mKv4.1 channel (Chabala and Covarrubias, 1993), and genes for these putative β-subunits have recently been cloned (Rettig et al., 1994; Scott et al., 1994).

2.1.5.5 Delineating the Binding Sites of Pharmacological Agents

Many of the pharmacological blockers discussed below are thought to bind within the ion conduction pathway and have therefore been used to probe the structure of the channel pore.

2.1.5.5.1 External TEA- and K^+-Binding Site

Kavanaugh et al. (1991, 1992) and MacKinnon and Yellen (1990) identified a tyrosine at the C-terminal end of the P-region (GYGDX*Y*PXX) of Kv channels that confers sensitivity to external TEA (TEA_e). Replacement of this tyrosine in rKv1.1 with valine (the equivalent residue in the TEA_e-insensitive rKv1.2) makes the channel resistant to TEA_e, and the reverse mutation in rKv1.2 makes the channel TEA_e sensitive (Kavanaugh et al., 1991). Similarly, introducing a tyrosine or phenylalanine in place of threonine in the *Shaker* channel greatly enhances the TEA_e sensitivity of the channel, and there is a direct relationship between the number of subunits containing tyrosine and degree of TEA_e block, suggesting that the four Kv subunits interact simultaneously with TEA to form a high-affinity binding site (Heginbotham and MacKinnon, 1992; Kavanaugh et al., 1992). Nine mammalian K^+ channels (Kv1.1, Kv1.6, Kv2.1-2.2, Kv3.1–Kv3.4, Kv5.1) contain this tyrosine. Five of these (Kv1.1, Kv3.1–Kv3.4) are extremely sensitive to TEA_e (K_d = ~0.1–1 m*M*), three (Kv1.6, Kv2.1–Kv2.2) are moderately sensitive (K_d = ~1–10 m*M*), and one (Kv5.1) has not been expressed (see Table 3). Clearly, the presence of tyrosine alone is not enough to make a channel highly TEA_e sensitive. The presence of hydrophobic residues (valine in Kv1.2, Kv4.1, Kv4.2, *Shal*, Kv6.1; alanine in *Shaw*) at the homologous position reduces the TEA sensitivity of channels, while positively charged residues (lysine in Kv1.4, arginine in Kv1.5) (Table 3) renders the channels TEA insensitive.

Channels with a positively charged residue at this position have an absolute requirement of external K^+ for channel opening (Pardo et al., 1992). For example, the rKv1.4 channel does not open in the absence of external K^+, but replacement of the lysine (GYGDM*K*PIT) with tyrosine allows the channel to open under such conditions (Pardo et al., 1992). The opening of Kv1.3 is similarly dependent on external K^+, presumably because the corresponding histidine is weakly positive under physiological conditions (Pardo et al., 1992). Kv1.5, the only other channel with a positively charged residue at this site (GYGDM*R*P; see Figure 12), has not been tested for its dependence on external K^+ for channel opening.

2.1.5.5.2 Internal TEA-Binding Site

Internal TEA (TEA_i) blocks Kv channels by interacting with a second site on the inner surface of the pore. Kv1.1, Kv1.3, Kv1.6, Kv2.1, and the *Shaker* channel are half-blocked by ~0.3 m*M* TEA_i (Taglialatela et al., 1991). This block is voltage dependent, becoming less potent at depolarized potentials.

The site of interaction of TEA_i is thought to lie ~20% within the cytoplasmic boundary of the membrane's electric field (Taglialatela et al., 1991; Kirsch et al., 1991b).

Mutagenesis studies have been used to localize the TEA_i binding site. Replacement of threonine (TM*T*TVGYG) with serine in the P-region of the *Shaker* channel markedly reduces TEA_i sensitivity (Yellen et al., 1991). The valine two residues downstream is also thought to participate in TEA binding (Kirsch et al., 1992a,b; Aiyar et al., 1993a). All the *Shaker*- and *Shab*-subfamily Kv channels have a valine in this position in place of the leucine present in mammalian *Shaw*- and *Shal*-subfamily channels (Figure 12). A Kv2.1/Kv3.1 chimera containing the Kv3.1 P-region (and therefore the leucine), is resistant to TEA_i, whereas a double reversion mutant (V369I + L374V) restores the sensitivity to TEA_i (Kirsch et al., 1992a). In the Kv3.1 channel, replacement of this leucine with valine makes the channel more sensitive to TEA_i (Aiyar et al., 1993b). These studies indicate that the P-region must extend at least partially through the cell membrane, and that residues within this segment must form part of the TEA_i binding site.

Residues in S6 also appear to interact with TEA_i. Mutation of a threonine in the S6 segment of *Shaker* (GVL*T*IAL) to serine reduced the sensitivity to TEA_i by about six-fold, and this residue also appears to participate in binding long chain alkyl derivatives of TEA (Choi et al., 1993).

2.1.5.5.3 Dendrotoxin-Binding Site

Peptide toxins [including dendrotoxin (DTX), charybdotoxin (CTX), and mast cell degranulation peptide (MCDP)] have been used to block channels with a high degree of selectivity and potency, and could be used as molecular calipers to probe the channel pore. DTX, a 59-amino acid peptide component of venom from the green mamba snake, *Dendroaspis angusticeps*, blocks Kv1.1 (K_d = 12–50 n*M*), Kv1.2 (K_d 3–4 n*M*), and Kv1.6 (K_d = 20–25 n*M*) with high affinity (Table 3). All the other cloned channels that have been studied (see Table 1) are resistant or only weakly sensitive to this toxin. Using site-specific mutational strategies, Hurst et al. (1991) have shown that the presence of an alanine and a glutamic acid (FAEAEE*AE*SH) in the loop linking S5 with the P-region in rKv1.1 confer sensitivity to DTX. The tyrosine associated with TEA binding also appears to affect the interaction of DTX with rKv1.1 (Hurst et al., 1991). It is important to determine whether the residues at the corresponding positions in Kv1.2 (FAEADE*RD*SQ), and Kv1.6 (FAEADD*VD*SL) underlie the DTX sensitivity of these channels.

2.1.5.5.4 Charybdotoxin-Binding Site

CTX, a toxin from the scorpion, *Leiurus quinquestriatus*, was originally described by Miller et al. (1985) as a specific blocker of calcium-activated K^+ channels, but is now recognized to also block voltage-gated K^+ channels (see Table 3). In early experiments CTX was reported to block the *Shaker* K^+ channel with high affinity, but more recent studies (Garcia-Calvo et al., 1992; Garcia et al., 1994) have shown that the inhibitory activity was due to contaminants present in some CTX preparations, and recombinant CTX does not block the *Shaker* channel. Similarly, studies with contaminated preparations initially suggested that Kv1.6 was CTX sensitive (Grupe et al., 1990), but subsequent experiments with recombinant CTX (Kirsch et al., 1991a) have not confirmed this finding (Table 3). The toxins that contaminated the earlier CTX preparations (agitoxins) have been purified to homogeneity, and shown to block *Shaker* channels with high affinity (Garcia-Calvo et al., 1992; Garcia et al., 1994). The channels most sensitive to recombinant CTX are Kv1.3 (K_d = 0.5–2 n*M*) and Kv1.2 (K_d = 1.7–22 n*M*), while all the other Kv channels that have been characterized are CTX insensitive (see Table 3).

Replacement of a glycine (residue volume ~60 Å^3) at the mouth of the CTX-sensitive Kv1.3 channel (PSS*G*FNSIPD) with larger residues such as glutamine or tyrosine (with volumes ranging from 145 to 195 Å^3) rendered the channel resistant to recombinant CTX, presumably by sterically hindering CTX entry into the outer vestibule; substitution with intermediate sized residues (e.g., aspartic acid or valine, with volumes ranging from 110 to 140 Å^3) reduced CTX sensitivity only moderately (R. Swanson, personal communication). Conversely, replacing phenylalanine at the homologous position in the CTX-resistant *Shaker* channel with the smaller glycine facilitated high-affinity binding between recombinant CTX and the channel, suggesting that the *Shaker* channel's resistance to CTX blockade is due to the narrow entry to its outer vestibule, rather than to a lack of CTX-binding sites (Goldstein and Miller, 1992). The CTX insensitivity of other channels (Table 3) may also be due to the presence of large residues at the entrance to the outer vestibule (Figure 12). Surprisingly, the identical position in the CTX-sensitive Kv1.2 channel (see Figure 2) is occupied by glutamine, which conferred resistance in the mutated Kv1.3 channel; this implies that compensatory differences at neighboring sites may make the opening to the outer vestibule wide enough for CTX to enter.

Studies with the contaminated CTX preparation had suggested that an aspartic acid at the N-terminal end of the pore region of the CTX-resistant *Shaker* protein (KSIP*D*AFWW) is involved in CTX binding (MacKinnon and Miller, 1989; MacKinnon et al., 1990). Surprisingly, neutralization of this aspartic acid in mKv1.3 (D to N, the identical mutation used in the *Shaker* channel) did not alter its sensitivity to recombinant CTX, implying that the toxin may interact differently with different channels (J. Aiyar, S. Grissmer, and K. G. Chandy, unpublished data). Similar studies with recombinant CTX have not yet been conducted on the CTX-sensitive Kv1.2 channel.

2.1.5.5.5 Mast Cell Degranulation Peptide-Binding Site

MCDP, a 22-amino acid peptide isolated from the honey bee, *Apis mellifera*, has been reported to affect both fast-inactivating (A-type) and slow-inactivating (delayed rectifier) channels (Stansfield et al., 1987; Brau et al., 1990). The channels most sensitive to MCDP are Kv1.6 (K_d = 10–200 n*M*), Kv1.1 (K_d = 45–490 n*M*), and Kv1.2 (K_d = 180 n*M*), all three of which are delayed

rectifiers (Table 3). The only cloned A-type channel that has been examined, Kv1.4, is MCDP resistant (Table 3). It is not yet known whether the other A-type Kv channels, Kv3.3, Kv3.4, and Kv4.2, are MCDP sensitive, nor have the residues that determine sensitivity to MCDP been delineated.

2.1.5.5.6 4-Aminopyridine-Binding Site

The classical K^+ channel blocker, 4-aminopyridine, blocks all known Kv channels with moderate potency (K_d ranging from 0.2–9 m*M*), Kv3.1 being most potently blocked. Recent experiments with Kv2.1/Kv3.1 chimeras suggests that the 4-AP-binding site is formed from the association of the N-terminal end of S5 and C-terminal-end of S6, which are both thought to lie in the inner vestibule of the channel pore (Kirsch et al., 1993; Yao and Tseng, 1993).

2.1.5.5.7 Ethanol and Halothane

Both these agents block the noninactivating fly *Shaw* channel at concentrations ranging from 70 to 170 m*M*, whereas other cloned channels are affected only at concentrations greater than 200 m*M* (Covarrubias and Rubin, 1993). While these experiments support the protein hypothesis for anesthetic action, the molecular sites of action of these drugs remain to be determined.

2.1.5.6 Experimentally Defining the Ion Conduction Pore

Since drugs that occlude the channel pore (TEA_e, TEA_i, DTX, and CTX) interact with residues in the loop linking the S5 and S6 segments, and in the P-region (see discussion above), it was initially suggested that the P-region contributed to the ion conduction pathway. To test this idea further, Hartman and colleagues (1991) exchanged the P-region of the low conductance rKv2.1 channel (8 pS) for that of the high conductance rKv3.1 channel (26–27 pS). The resulting Kv2.1/Kv3.1 chimera had all of the pore properties of the donor Kv3.1 channel. Additional mutational experiments identified residues that were purported to determine ion selectivity (Kirsch et al., 1992a,b; Yool and Schwarz, 1991; DeBiasi et al., 1993a,b). Collectively, these studies indicated that all or most of the pore determinants were contained in the P-region. This conclusion is further supported by the fact that the P-region is the only recognizably conserved segment in all K^+-selective channels (see Figure 15).

Recent studies suggest that the S6 segment and the loop linking the S4–S5 segments also contribute to the formation of the ion conduction pathway (Kirsch et al., 1993; Lopez et al., 1993). Choi et al. (1991) showed that mutations in the S6 segment of the *Shaker* channel altered binding to alkyltriethylammonium blockers, which implied that this region lined part of the inner surface of the channel pore. More recently, Lopez et al. (1993) were able to transfer most of the pore properties of the rKv3.1 channel to the *Shaker* channel by transplantation of the rKv3.1-S6 segment, but not the P-region; only sensitivity to external TEA was transferred by the P-region transplant. Lastly, we have recently found that a mutation in the S6 segment of mKv3.1 (M430L) altered single channel properties (i.e., induced a channel flicker that was dependent on the permeant ion) without affecting sensitivity to internal TEA, although the converse mutation (L427M) in mKv1.3 did not produce any change (J. Aiyar, S. Grissmer, and K. G. Chandy, unpublished data). Mutations in the S5–S5 linker of the *Shaker* channel have also been reported to change the channel's sensitivity to TEA_i and to alter its single channel properties (Isacoff et al., 1991; Slesinger et al., 1993). Thus, the P-region along with the S6 segment and the S4–S5 linker appear to contain most of the pore determinants. In fact, the model for K^+ channels proposed by Durell and Guy (1992) predicts an ion conduction pathway comprised of these three regions.

We would emphasize, however, that considerable caution must be exercised in interpreting all such mutational experiments. For one thing, it is clear that an amino acid substitution in any protein may have distant as well as local consequences. While many of the results of P-region mutagenesis have been dramatic and compelling, the fact that a particular substitution affects ion selectivity, for example, is not positive proof that this residue interacts directly with the permeant ion. As a result of such distant interactions, the functional properties of a particular residue in one Kv channel may be quite different from its properties at the same position in chimeras or other channels. For example, a valine/leucine exchange in the P-region of rKv2.1, or that of a Kv2.1/Kv3.1 chimera containing the Kv3.1 P-region, have different consequences from the identical mutation in native Kv3.1. Replacing a valine in the P-region of rKv2.1 (ITMTT*V*GYG) with leucine alters single channel properties, and so does the converse leucine to valine mutation in the Kv2.1/Kv3.1 chimera (Kirsch et al., 1992a,b). The leucine to valine mutation in native Kv3.1, however, does not change its single channel properties (Aiyar et al., 1993b). These conflicting results are most likely due to differences in the interactions between the leucine (or valine) and residues outside the P-region.

2.1.6 Conclusion

Potassium channels are the largest and most diverse known family of ion channels, and the voltage-gated K^+ channels constitute the largest group among these. The explosion of cloned genes and cDNAs over the past few years has led to an extraordinary accumulation of information on their functional diversity, gene organization, tissue-specific expression, and structure–function relationships, information that we have attempted to organize in this review.

Over the next few years we expect to witness major advances in our knowledge of K^+ channels, advances of both basic biological and practical significance. The availability of high-level expression systems for Kv channels should facilitate the biochemical characterization of these proteins, identifying the processes involved in their synthesis and assembly, and the variety of posttranslational modifications that they undergo. Such systems should also be capable of providing purified protein of sufficient quality and

quantity for direct structural analysis, and the simple schematic diagrams that now constitute our best images of Kv channels may finally be replaced by empirically determined structures.

Identification of those genetic elements and mechanisms that regulate Kv gene transcription will provide a rationale for understanding their complex tissue-specific expression, at the same time explaining the surprising heterogeneity of Kv transcripts and their peculiarly long 5′ and 3′ untranslated regions. Resolution of these issues will also help in understanding the selective forces that have resulted in the expansion of the Kv subfamilies in vertebrates, and the striking lack of introns in the protein-coding regions of most of the *Shaker* subfamily genes.

While therapeutic drugs affecting other ion channels have long been known, Kv channels are just beginning to be developed as drug targets. The great diversity of these channels, and their involvement in myriad cellular functions, should provide the possibility of developing highly selective agents for the treatment of diseases affecting both electrically excitable and nonexcitable tissues. Finally, establishing associations between genetically determined diseases and mutations in specific Kv genes will forge additional links in the chain connecting basic biological knowledge with the treatment of human disease.

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